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# Progress in energy from microalgae: A review

Ali Bahadar [a], M. Bilal Khan [b,*]

[a] School of Chemical and Materials Engineering, National University of Sciences and Technology, H-12 Sector, Islamabad, Pakistan
[b] Centre for Energy Systems, USAID Centre for Advanced Studies, National University of Sciences and Technology, Sector H-12, Islamabad, Pakistan

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Microalgae have great potential as renewable fuel sources, but a dire need exists for high-level academic and industrial research into their growth and bioprocessing. New algae strains that efficiently use $CO_2$ and wastes as nutrients, novel oil extraction methods, and industrial-scale designs for fuel production are imperative for long-term energy sustainability. A particular challenge to research in this field is the transition from pilot studies to industrial operations, which often exposes algae cells and their products to hostile environments, reducing yields. Hence, a need exists to integrate algae cell engineering with predictive bioprocess engineering to ensure economic and environmental feasibility and minimize the number of full-scale trials that fail. This review provides a brief overview of biofuel production from microalgal biomass. It highlights the most promising microalgae species for different types of fuel, the proper choice of photobioreactor and process parameters, product extraction techniques, and the main biofuel products. The main goal of this paper is to promote research into energetically- and environmentally-favorable technologies via the development of better algal strains and separation, extraction, and conversion methods.



**Contents**



* Corresponding author. Tel.: +925 190 855 100.
*E-mail address:* principal.ces@nust.edu.pk (M. Bilal Khan).

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## 1. Introduction

Declining petroleum reserves, increasing fuel demands for commuting and power generation, and environmental problems have necessitated the search for sustainable sources of energy. Liquid biofuels have received much attention in this context. Numerous biomass feedstocks, including both terrestrial plants and aquatic algae, have been reported to produce renewable fuels to replace fossil fuels and mitigate carbon dioxide ($CO_2$) emissions [1]. Marine biomass has the potential to provide both food and fuel at productivity levels per unit area that match or exceed those of terrestrial crops [2]. Among the available feedstocks, aquatic microalgae are ideal for producing liquid fuels [3]. Their rapid growth, high biomass yields, product diversity, and ease of harvest from ponds or closed photobioreactor systems give them excellent commercial potential as sustainable environmentally-friendly carbon-neutral fuel sources [4,5].

Microalgae are photosynthetic microorganisms from either marine or freshwater environments and can include bacteria (Cyanobacteria), diatoms (Chromalveolata), other protists (e.g., Chromista), and unicellular plants (e.g., Chlorophyta). They exist as individual cells or as chains of cells but do not form differentiated, multicellular organisms, as do macroalgae. Some species contain more than 70% lipids (dry weight basis) [6], and they grow exponentially under optimal conditions. They have been investigated because of their high photosynthetic rates (e.g., $6.9 \times 10^4$ cells/mL/h) [7] and efficiency, with biofuel yields up to 12,000 L/ha, which is much higher than terrestrial plants [8,9]. Microalgae currently cost more to cultivate than traditional crops and yield lower profits, but they remain a promising tool for future energy needs because they occupy less area than other energy crops, like *Jatropha* (Euphorbiaceae) or *Pongamia* (Fabaceae) [10,11].

The high $CO_2$ and nutrient demands of microalgae can be met using flue gases and waste water from other industrial processes, providing ecological benefits while lowering the cost of biomass production. Some microalgae can also synthesize desirable compounds, like $\beta$-carotenoids, docosahexaenoic acid (DHA), and astaxanthin, with commercial or pharmaceutical applications [6,12,13]. Moreover, microalgae have shown great potential to produce a wide spectrum of fuel products in pilot studies: (1) hydrogen ($H_2$) via direct and indirect biophotolysis, (2) biodiesel through transesterification, (3) biomethane via anaerobic digestion, (4) bioethanol by fermentation, (5) bio-oil via thermochemical conversion, and (6) green diesel and gasoline through direct catalytic hydrothermal liquefaction [14–17]. However, the transition from small-scale to industrial-scale operations often exposes algae cells to hostile conditions, with consequent declines in product yields. The recovery and concentration of algae from highly dilute suspensions, in particular, requires steps that can prematurely lyse cells and reduce extract yields. Therefore, an urgent need exists to integrate the best algae cell and bioprocessing engineering methods to ensure economic and environmental feasibility and to minimize the number of full-scale trials that fail. Conditions for a technically and economically viable biofuel resource are that it should be competitive or cost less than petroleum fuels; should require low to no additional land use; should enable air quality improvement (e.g., $CO_2$ sequesteration); and should require minimal water use [18]. Production cost associated with microalgae biofuel is the major barrier in its commercialization but still fuels from algae are promising, as they may already be viewed as competitive with petroleum fuels, if the full environmental impact of the latter types of fuels is taken into consideration. Issues of climate change may force us to move beyond petroleum long before it runs out [19,20]. A significant number of startup companies are making attempts to commercialize algae fuels. The table in Appendix A shows a list of 43 companies [19,21–23] which are actively participating in the development of algal fuels. Singh [21] presented the region wise percentage of companies around the world producing algae fuels. Most of the companies contributing to development of algal biofuel are based in America i.e. 78%, 13% are Europe based and 9% are from other regions. There are many research groups, companies and governments involved in the commercialization of algae based biofuels. The Department of Energy (DOE) currently spends about $85 million on 30 research projects to develop algal biofuels. Obama committed another $14 million to the idea. According to Piccolo [24] the biggest algae investment in the EU is the £26 million publically funded project by the UK Carbon Trust which planned to build a large algae farm in Northern Africa [25]. In another development, a Spanish renewable energy company Aurantia and Green Fuel Tech of Massachusetts (USA) formed a partnership through a $92 million project in 2007 to produce algae oil. In the long run, this project will target to scale up to 100 ha of algae greenhouses, producing 25,000 t of algae biomass per annum. The plant will obtain its $CO_2$ from a cement plant near Jerez in Spain. In yet another endeavor, an Italian energy company, Eni, has installed a 1 ha pilot facility for algae oil production in Gela, Sicily. This project is testing the photobioreactor facility as well as open ponds [21,24].

This review provides an overview of recent progress in algal energy production, summarizing the most promising algal strains, cultivation methods, harvesting and extraction techniques, and conversion technologies for the commercial application of microalgae. We provide a critical analysis of the potential of selected microalgal strains and processes to yield biofuels. This review does not consider multicellular macroalgae, which are more difficult to grow in bioreactors, yield fewer products at lower concentrations, and have not yet been researched as thoroughly as microalgae [26,27]. Furthermore, microalgae have less complex structures, faster growth rates, and higher oil contents than do macroalgae.

## 2. Biofuel algae strains and their culture

Like most other photosynthetic organisms, microalgae use light energy to convert $CO_2$ into organic compounds. Microalgae are more photosynthetically efficient than higher plants [28]; thus

**Table 1**
Microalgae strain cultivation in optimized cultures.

| Algae strain (% lipid content) | Light intensity (μmol/m$^2$/s) | Temp | Medium | $CO_2$ | pH | Yield | Reference |
|---|---|---|---|---|---|---|---|
| ***Botryococcus braunii* 765 (29–75%)** | 150 ± 10 | 25 °C | BG11 medium (per liter): 1500 mg $NaNO_3$, 40 mg $K_2HPO_4 \cdot 3H_2O$, 75 mg $MgSO_4 \cdot 7H_2O$, 36 mg $CaCl_2 \cdot 2H_2O$, 6 mg $C_6H_8O_7 \cdot H_2O$, 6 mg $Fe(NH_4)_3C_{18}H_{10}O_{14}$, 1 mg $Na_2 \cdot EDTA$, 20 mg $Na_2CO_3$, 2.86 mg $H_3BO_3$, 1.81 mg $MnCl_2 \cdot H_2O$, 0.222 mg $ZnSO_4 \cdot 7H_2O$, 0.079 mg $CuSO_4 \cdot 5H_2O$, 0.39 mg $Na_2MoO_4 \cdot 2H_2O$, 0.049 mg $Co(NO_3)_2 \cdot 6H_2O$ | 20% $CO_2$<br>10% $CO_2$<br>5% $CO_2$<br>2% $CO_2$ | 6.3<br>6.5<br>6.8<br>7.0–7.5 | 2.31 g/L biomass on day 25 with 12.71% (w/w) lipid content<br>1.61 g/L on day 24 with 12.44% lipid content<br>1.69 g/L on day 26 with 11.21% lipid content<br>2.18 g/L on day 28 with 10.41% lipid content | [32] |
| ***Schizochytrium limacinum* SR21 (ATCC MYA-1381) (50–77%)** | – | 25 °C | Artificial seawater containing (per liter): 18 g NaCl, 2.6 g $MgSO_4 \cdot 7H_2O$, 0.6 g KCl, 1.0 g $NaNO_3$, 0.3 g $CaCl_2 \cdot 2H_2O$, 0.05 g $KH_2PO_4$, 1.0 g Trizma base, 0.027 g/L $NH_4Cl$, $1.35 \times 10^{-4}$ g vitamin $B_{12}$, 3 mL chelated iron solution, and 10 mL PII metal solution (boron, cobalt, manganese, zinc, and molybdenum) plus 10 g/L glucose, 1 g/L yeast extract, and 1 g/L peptone | – | 7.5–8.0 | 37.90 g/L maximum biomass yield<br>3.25 g/L/d maximum biomass productivity 1<br>73 mg/g dry wt DHA[a] content<br>6.56 g/L DHA yield | [33,34] |
| ***Nannochloropis* sp. (31–68%)** | 300 | 26–27 °C | (per liter): 1.45 g $KNO_3$, 0.12 g $KH_2PO_4$, 0.04 g $NaHCO_3$, 1 mL Fe-EDTA solution (240 mg $FeCl_3 \cdot 4H_2O$ in 100 mL 0.05 M $Na_2$-EDTA), 1 mL trace element stock solution (0.01 g $CuSO_4$, 0.022 g $ZnSO_4$, 0.01 g $CoCl_2 \cdot 6H_2O$, 0.18 g $MnCl_2 \cdot H_2O$, and 0.006 g $Na_2MoO_4 \cdot 2H_2O$, in 1 L distilled water) | 1.5% $CO_2$ at aeration rate of 0.31 $min^{-1}$ $L^{-1}$ | 7–8 | Optimum cell density (for a 10-cm light path reactor outdoors) is $5.5 \times 10^8$ cells/mL | [35] |
| ***Nitzschia laevis* UTEX 2047 (69.1%)** | | 20 °C | Batch-fed (based on glucose feedback) continuous supplemented LDM[b] medium (per liter): 1 g tryptone, 892 mL artificial seawater, 100 mL Bristol solution, 6 mL PIV metal solution, 1 mL of $25.0 \times 10^{-5}$ g/L biotin, 1 mL of $15.0 \times 10^{-5}$ g/L vitamin B12, supplemented with 5 g/L glucose and 30 mg/L $Na_2SiO_3 \cdot 9H_2O$ | – | 8.2 | 3.14% dry wt EPA[c] content<br>695.2 mg/L EPA yield<br>49.7 mg/L/d EPA productivity | [36] |
| ***Chlorella vulgaris* (56%)** | 76 | 25 °C | Low nitrogen medium: 203 mg/L $(NH_4)_2HPO_4$, 2.236 g/L KCl, 2.465 g/L $MgSO_4$, 1.361 g/L $KH_2PO_4$ and 10 mg/L $FeSO_4$ | – | 6.0 | Biphasic growth rate: 0.69 g/d for days 1–4, 0.12 g/d after day 4<br>$7.0 \times 10^6$ cells/mL/d maximum<br>58% dry wt lipid content<br>28 kJ/g caloric value<br>24 mg/L/d biomass productivity | [37] |
| ***Chlorella emersonii* (63%)** | 76 | 25 °C | Low nitrogen medium: 203 mg/L $(NH_4)_2HPO_4$, 2.236 g/L KCl, 2.465 g/L $MgSO_4$, 1.361 g/L $KH_2PO_4$, and 10 mg/L $FeSO_4$ | | 6.0 | 0.38 g/d growth rate<br>$4.0 \times 10^6$ cells/mL/d maximum<br>34% dry wt lipid content<br>24 kJ/g caloric value<br>36 mg/L/d biomass productivity | [31] |
| ***Chlorella minutissima* UTEX2341 (57%)** | 50 | 25 °C | Basic Medium (BM=N8Y medium, per liter distilled water): 1 g $KNO_3$, 0.74 g $KH_2PO_4$, 0.207 g $Na_2HPO_4$, 0.013 g $CaCl_2 \cdot 2H_2O$, 0.01 g FeNaEDTA, 0.025 g $MgSO_4$, 0.1 g yeast extract, and 1 mL micronutrient solution (3.58 g $Al_2(SO_4)_3 \cdot 18H_2O$, 12.98 g $MnCl_2 \cdot 4H_2O$, 1.83 g $CuSO_4$ $5H_2O$, 3.2 g $ZnSO_4 \cdot 7H_2O$ per liter of distilled water). Added carbon sources (per liter): 1 g dextrose, 1.46 g oxalic acid, 0.88 g starch, 0.93 g sucrose, 1.22 g glycine, 1.33 g sodium acetate, and 1 g glycerin with 0.39 g carbon/L | Atmospheric $CO_2$ source was not used | – | 1.78 g/L/d biomass<br>16.11% lipid content<br>286.76 mg/L/d lipid productivity<br>180.68 mg/L/d FAME[d] productivity | [38] |
| ***Chlorella protothecoides* UTEX 256 (23/55%)** | – | 28 °C | Grown on crude glycerol. Basal culture medium (per liter): 0.7 g $KH_2PO_4$, 0.3 g $K_2HPO_4$, 0.3 g $MgSO_4 \cdot 7H_2O$, 25 mg $CaCl_2 \cdot H_2O$, 25 mg NaCl, 3 mg $FeSO_4 \cdot 7H_2O$, 0.01 mg vitamin B1, and 1 mL A5[e] solution | – | 6.8 | 3.9 g/L/d biomass productivity<br>0.62 g/g CDW[f] lipid content<br>2.4 g/L/d lipid productivity | [39] |
| | 270 | 25 °C | | | 7.5 | | [40] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***Neochloris oleoabundans* strain 1185 (35–65%)** | | | Medium (per liter): 0.91 mM $MgSO_4 \cdot 7H_2O$, 8.82 mM $NaNO_3$, 0.43 mM $KH_2PO_4$, 1.29 mM $K_2HPO_4$, 0.43 mM NaCl, 0.17 mM $CaCl_2 \cdot 2H_2O$, 30.7 mM $ZnSO_4 \cdot 7H_2O$, 7.3 mM $MnCl_2 \cdot 4H_2O$, 4.9 mM $MoO_3$, 6.3 mM $CuSO_4 \cdot 5H_2O$, 1.7 mM $CoNO_3 \cdot 6H_2O$, 0.185 mM $H_3BO_3$, 0.171 mM EDTA, 0.553 mM KOH, 18 mM $FeSO_4 \cdot 7H_2O$, 10.2 mM $H_2SO_4$ | Gas injection($CO_2$ and air), flow rate of 0.5 L/min | | 16.5 $g/m^2/d$ biomass area productivity<br>23% dry wt total lipid content<br>3.8 $g/m^2/d$ total lipid productivity | |
| ***Parietochloris incisa* (62%)** | – | 26 °C | Bold Basal Medium (BBM) (per liter): 1.5 g $NaNO_3$, 0.075 g $K_2HPO_4$, 0.175 g $KH_2PO_4$, 0.075 g $MgSO_4 \cdot 7H_2O$, 0.084 g $CaCl_2 \cdot 2H_2O$, 0.00498 g $FeSO_2 \cdot 7H_2O$, 0.05 g EDTA · 2Na salt, 0.025 g NaCl, 0.031 g KOH, 11.42 μg $H_3BO_3$, 1.44 μg $MnCl_2 \cdot 4H_2O$, 8.82 μg $ZnSO_4 \cdot 7H_2O$, 1.57 μg $CuSO_4 \cdot 5H_2O$, 0.049 μg $Co(NO_3)_2 \cdot 6H_2O$, and 0.71 μg $MoO_3$ | 1% $CO_2$ (v/v) at rate of 1 L/min | – | 36.32 mg/L/d lipid productivity<br>523.19 mg/L volumetric yield | [41] |

[a] DHA, docosahexaenoic acid.
[b] LDM, Lewin's marine diatom medium.
[c] EPA, eicosapentaenoic acid.
[d] FAME, fatty acid methyl ester.
[e] A5 solution: 286 mg $H_3BO_3$, 250 mg $MnSO_4 \cdot 7H_2O$, 22.2 mg $ZnSO_4 \cdot 7H_2O$, 7.9 mg $CuSO_4 \cdot 5H_2O$, 2.1 mg $Na_2MoO_4 \cdot 2H_2O$ and100 mL $dH_2O$.
[f] CDW, cell dry weight.

they are important potential sources of biodiesel and can be used to mitigate the industrial production of $CO_2$. Under normal growing conditions, microalgae yield a caloric value of 18–21 kJ/g/d. However, the optimal $CO_2$ concentrations, nutrient sources, and biofuel yields must be investigated for each strain of microalga [29]. For example, lipid production in *Chlorella emersonii* could be as high as 58–63% dry weight when grown with low nitrogen levels, and *C. protothecoides* contained 55% lipid (dry weight) when grown heterotrophically with corn powder hydrolysate under nitrogen limitation [30]. Furthermore, the high production costs of microalgal biofuels can be mitigated by selecting the best strains and by developing process technologies that are economically viable at industrial scales [31]. In this section, we discuss some of the most economically-promising microalgal strains, their cultivation conditions, and their yields. All example strains are summarized in Table 1.

*Botryococcus braunii* 765 (Plantae: Chlorophyta) is a green colonial microalga that can yield biodiesel, hydrocarbons, and biocrude oil. Typically, these algal cells are incubated in laboratory bioreactors at 25 °C under $150 \pm 10$ μmol/$m^2$/s light for 2 weeks before being transferred to an enclosed photobioreactor (see Section 3) containing sterile modified BG11 medium. The effects of $CO_2$ concentrations (from 2% to 20%) on the growth rate of *B. braunii* were studied. Maximum biomass production occurred with 20% $CO_2$, giving a biomass yield of 2.31 g/L that was 12.71% (w/w) lipids on day 25 [32]. This shows that hydrocarbon content increases as the concentration of $CO_2$ increases and by adding 2% sodium hypochlorite in photobioreactors.

Strains of *Schizochytrium* (Chromalveolata: Heterokontophyta) have excellent production rates on the order of 7.3–9.4/day. Biomass densities of some strains reach 200 g/L within fermentation cycles of 90–100 h under nitrogen- and glucose-fed cultures [33]. *Schizochytrium* can also ferment glycerol to produce DHA. Crude glycerol is a major byproduct of biodiesel production. DHA yield significantly increases to 4.91 g/L by adding 1 g/L ammonium acetate at 19.2 °C. However, glycerol concentrations above 25–35 g/L decrease cell growth. At 35 g/L glycerol, cellular lipid content reached 73.3% [34].

Algal growth and productivity are highly affected by the light path (LP), which can range from 1.3 to 17 cm in vertical glass photobioreactors. Shorter LPs (e.g., 10 cm) resulted in higher mass productivity of *Nannochloropsis* sp. (Chromista: Ochrophyta) [35]. The two main factors that affect reactor efficiency are the total illuminated surface area and culture volume. The lower these values, the more efficient and cost effective the reactor. Open raceway ponds are more productive per unit illuminated area than flat-plate reactors, but they require six times more volume than closed photobioreactors to produce the same amount of algae.

To increase the productivity of the diatom *Nitzschia laevis* (Chromista: Ochrophyta), a continuous fed-batch process was developed [36]. Glucose, tryptone, nitrates, and yeast extract were considered essential media components to increase productivity. The optimum ratio of 31:1 glucose: nitrate increased cell growth yield to 22.1 g/L; these conditions also increased eicosapentaenoic acid (EPA) production.

For fuel use, microalgae must have high caloric value and biomass productivity. The microalgae *C. vulgaris* and *C. emersonii* (Plantae: Chlorophyta) were cultivated in a pumped tubular photobioreactor to compare low-nitrogen and Watanabe's media [37]. Using the low-nitrogen medium increased both lipid content and caloric value for both species, but the biomass productivity of *C. vulgaris* was higher in Watanabe's medium (40 mg dry wt/L/d) than in the low-nitrogen medium (24 mg dry wt/L/d).

*C. minutissima* UTEX2341 is a promising source of biodiesel, because it contains C16 and C18 lipids, which are components of diesel oil. When cultivated in a flask receiving 50 μmol $m^2$/s light

in organic carbon medium, its biomass productivity was 1.78 g/L/d. To increase its biomass productivity, glycerin can be used as an organic carbon source and casein as a nitrogen source [38].

Crude glycerol has been considered as an important alternative carbon source to glucose for the cultivation of microalgae. It is a byproduct during biodiesel production, so using it as a carbon source makes the cultivation process more cost-effective and eliminates its disposal cost. Strains of *C. protothecoides* cultivated in continuous fed-batch mode with glycerol as the carbon source had higher maximum lipid productivity (3.9 g/L) than in batch mode. Another advantage of fed-batch cultivation is that it can use glycerol with 62% purity with similar productivity to the use of pure glycerin in batch mode [39].

*Neochloris oleoabundans* (strain 1185) (Plantae: Chlorophyta), a freshwater microalga, is known for its ability to store lipids and triacylglycerides (TAG). However, the choice of nutritional medium did not affect TAG productivity [40].

*Parietochloris incise* (Plantae: Chlorophyta) is a potential source of polysaturated fatty acids (PAC) and arachidonic acid (AA). It was cultivated in a vertical tubular photobioreactor to optimize its nutrient proportions and productivity to improve its cost effectiveness. Nitrogen starvation decreased AA productivity; the critical concentration was 0.5 $g^{-1}$ sodium nitrate, giving 36.32 mg/L/d lipid productivity and 523.19 mg/L volumetric yield. In general, phosphorus and nitrogen starvation seem to enhance AA productivity more than nitrogen starvation alone [41].

A primary strategy for most algal biofuel producers is to identify the algal species that have a high oil content, that will also grow quickly to produce biodiesel, biocrude and drop-in fuels. Algae with high oil content such as *B. braunii* (Bb) grow slowly and can be harvested only a few times a week, whereas algae with lower oil content such as *Dunaliella* or *Nannochloropsis* (in the 20–40% range) will grow more quickly and can be harvested daily or a few times a day. For this reason, most algal R&D projects and pre-commercial projects are using algal strains with 20–40% content [42].

## 3. Algae cultivation

Microalgae can be cultivated in different types of systems, mainly in open ponds or raceways and in enclosed photobioreactors. Open cultures are usually located outdoors and rely on direct sunlight, while closed photobioreactors can be either indoors or, preferably, outdoors to use free sunlight [43]. Most species of microalgae can be grown in photobioreactors, while open systems are more limited.

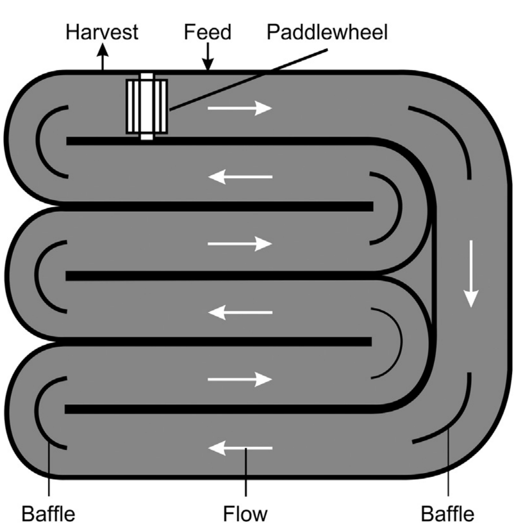



**Fig. 1.** Schematics of a raceway pond.

### 3.1. Cultivation in ponds and raceways

Open ponds are simple cultivation tanks that are largely obsolete, having been replaced by more efficient raceway ponds. Raceway ponds as shown in Fig. 1 form closed circuits approximately 0.25 m wide and 0.4 m deep through which the water is circulated using a paddle wheel [44]. They are shallow to maximize light penetration. Where land and water are inexpensive, raceways are extremely cost effective to construct and operate. They require no cooling and do not experience oversaturation with oxygen, which can threaten biofuel production in closed systems. However, they have lower productivity per unit area and volume than closed photobioreactor systems because of the low light-to-volume ratio [45]. Additionally, these systems can be easily contaminated by other microorganisms that can compete with the cultivated algal strain [46,47]. They must be kept highly alkaline to prevent contamination; this high pH limits their suitability to only a few species. The possibility of contamination is often cited as a serious limitation of open systems [5] and it is true that most of the species cultured in such systems currently do grow in selective environments, i.e. *Arthrospira* (*Spirulina*) [high alkalinity], *Dunaliella salina* [high salinity], and *Chlorella* [high nutrients] [48,49]. However, other species with 'normal' growth requirements have also been grown successfully in open ponds [5], either in batch mode [e.g., *Haematococcus pluvialis*] [50], or continuously for very long periods [e.g., *Phaeodactylum tricornutum*, *Nannochloropsis* and *Pleurochrysis carterae*] without significant contamination problems [5]. Odlare [51] studied the cultivation of algae in open pond (*Lake Mälaren*) in Sweden. The idea behind this research was to enhance indigenous algae production rather than inoculate new species into the system. The production rate of biodiesel from algae was estimated using data from Weyer [52], Amin [53] and Chisti [54] for the estimation of potential for using algae as energy source in the Mälardalen region. The biomass productivity was enhanced by nutrient addition by using Jaworskis Medium (JM). The estimate of the potential of algae to replace vehicle fuels in the Mälardalen region shows that an area corresponding to at least 40% of the Mälaren would be required to satisfy current demand [51].

### 3.2. Cultivation in photobioreactors

Closed photobioreactors are designed to overcome the limitations of open pond systems [55]. They have higher efficiency and biomass productivity, shorter harvest times, high surface-to-volume ratios, reduced contamination risks, and can be used to cultivate a greater range of algal species than open systems [29,54]. Additionally, they can use wastewater or flue gases from power plants, providing additional environmental benefits [56]. However, they are much more expensive to construct than open systems.

Closed photobioreactors involve a thin panel of transparent tubes or plates placed horizontally or vertically and provided with $CO_2$ cylinders. There are several types, including tubular, flat plate, column, and biofilm photobioreactors. They can be airlift, flat inclined, bubble column, column aeration, solar penthouse-roof, and multistage continuous flow photobioreactors. Table 2 summarizes some important process variables and specifications for photobioreactor designs used for different microalgae species.

Tubular photobioreactors as shown in Fig. 2 are the most widely used and considered the most promising, because they yield high biomass and have short harvest times [41]. They comprise parallel tubes (0.2 m in diameter or less) that are positioned horizontally or vertically to maximize sun exposure.

**Table 2**
Cultivation of microalgae species in closed photobioreactor systems.

| Type of photobioreactor (PBR) | Algae taxon | Specifications | Size and capacity | pH | Solar irradiance | Standardization | Limiting temp. | Temperature control | Biomass concentration during production | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Airlift | *Botryococcus braunii* | Tubes: 10 cm diam., 50 cm long | Capacity = 3 L | 6.0–8.0 | $120\times10^{-6}$ mol/m$^2$/s | Difficult | 25 °C | In cabinet (25 °C) | 2.31 g/L max | [32] |
| Airlift | *Chlorella* sp. | Acrylic glass tubes | SA = 1.337 m$^2$ | _ | 350 μmol/m$^2$/s at surface of reactor from 16 cool white 40 W lamps | Commercial | 25 °C | 25 ± 2 °C | 0.21 g/L/d | [63] |
| Airlift | *Chlorella vulgaris* | Acrylic glass columns: 10 cm diam., 25 cm high | Capacity = 2 L | 8.0–10.0 | _ | Possible | 30 °C | _ | 0.89–0.28 g/L/d | [68] |
| Biofilm photobioreactor | *Botryococcus braunii* | A biofilm growth surface (8 mm thick concrete layer) | SA = 0.275 m$^2$ Capacity = 0.60 L | 8.3 | | Cultivates algae as a biofilm with reduced energy and water requirement | 25 °C | Transparent film capable of blocking infrared radiation | 0.71 g/m$^2$/day | [64] |
| Bubble column | *Aphanothece microscopica* | Glass tube: 7.5 cm diam., 4 mm thick, 75 cm high | Capacity = 3 L; 1.5-cm diam. air diffuser in center of column | _ | 4.25–6.50 kW/m$^2$/day | _ | 35 °C | _ | 0.770 g/L/d | [61] |
| Bubble column | *Isochrysis* aff. *Galbana* | _ | Capacity = 5 L | 8.2 | $430\pm30\times10^{-6}$ mol/m$^2$/s | Difficult | _ | 22.0 ± 0.1 °C | _ | [70] |
| Bubble column | *Porphyridium purpureum* | Glass bubble-column tubes, 0.4 m high, 0.1 m diam. | SA = 0.1096 m$^2$ | _ | 120 μE/m$^2$/s$^1$ | _ | 25 °C | Transparent jacket with thermostat unit (25 °C) | _ | [65] |
| Flat plate | *Chlorella vulgaris* | Acrylic glass | SA = 0.084 m$^2$, capacity = 1.5 L, 3.0 L | 6.8 ± 0.1 | $980\pm80\times10^{-6}$ E/m$^2$/s | Possible | 30 °C | Cooling jacket located at front of reactor (29 °C) | 0.027–0.045 g/L/h | [71] |
| Flat plate | *Dunaliella tertiolecta* | Polycarbonate: 0.01 m thick; plates $0.61\times0.61\times0.1$ m$^3$ | Capacity = 30 L | 7.6 | Optical density was measured at 806 nm | Commercial | 22.5 °C | Acrylic water basin (40 L, ≈10 cm high) with type *K* thermocouples (26–35 °C, accurate to ± 0.3 °C) | 3.42 ± 0.39 g/d | [72] |
| Flat plate | *Spirulina platensis* | Glass tank: 90 cm length, 2.6 cm internal width, 70 cm high | $\theta$ = 30° (summer), 60° (winter) | 9.5 | 10% Of incident irradiance | _ | 35 °C | Water sprinklers along top of front panel | _ | [69] |
| Flat plate | *Synechocystis aquatilis* | Three plates (1000 m, 1000 m, and 360 m) separated by 0.5 m in a glass green house | SA = 3.5 m$^2$ | _ | 1–12 MJ/m$^2$/d | _ | _ | _ | 30 g/m$^2$/d | [67] |
| Multistage continuous flow | *Scenedesmus* sp. | Standard window glass: 0.25 in. thick, six flat plate 2.1 m long, 0.5 m high, 0.15 m deep | Capacity = 900 L | _ | 750 nm light | Commercial | 35 °C | Evaporative cooling (simple, economical) (25–35 °C) | _ | [73] |
| Stirred tank | *Spirulina maxima* | Acrylic glass | _ | 10.3–11.4 | _ | _ | 30 °C | Roux bottles (31 °C) | 10.8 g/m$^2$/d | [74] |
| Tubular | *Haematococcus pluvialis* | Parallel plastic tubes, diam. = 0.18–0.41 m | SA = 100 m$^2$; capacity = 25,000 L | _ | _ | Difficult | 20 °C | PBR flooded with cold sea water (5–25 °C) | 13 g/m$^2$/d | [75] |
| Tubular | *Phaeodactylum tricornutum* | Acrylic glass tubes; 4-m tall airlift section with degasser zone; riser/downcomers 0.053 m diam., 0.007 m thick | Tube length = 80 m; surface area (SA) = 12/m$^2$; degasser zone length = 0.22 m; $\theta$ = 60°; reactor volume = 0.2 m$^{3.}$ | 7.7 | Saturation irradiance = $185\times10^{-6}$ E/m$^2$/s | Difficult | 35 °C | PBR immersed in pond water (20 ± 2 °C) | 1.5 g/L/d. | [59] |

**Table 2** (*continued*)

| Type of photobioreactor (PBR) | Algae taxon | Specifications | Size and capacity | pH | Solar irradiance | Standardization | Limiting temp. | Temperature control | Biomass concentration during production | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Tubular | *Phaeodactylum tricornutum* | Plastic tubes: 106 m length, 0.03 m diam.; arranged around a circular frame, 1.2 m diam., 0.8 m high | Capacity=75 L; height of degasser above helical loop=2 m | 7.7 | Saturation irradiance= $200\times10^{-6}$ E/m²/s; average irradiance= $250\times10^{-6}$ E/m²/s | Difficult | 35 °C | Heat exchanger (28 °C) | 1.5 g/L/d | [47] |
| Tubular | *Phaeodactylum tricornutum* | Tubes: 0.03–0.06 m diam. | – | 7.7 | – | Commercial | – | – | – | [76] |
| Tubular | *Porphyridium cruentum* | Tubes 0.064 m diam., 1500 m total length | Capacity=6 m³ | – | – | Commercial | – | – | 35 g/m²/d | [43] |
| Tubular | *Spirulina platensis* | Glass tubes: 24 m long, 48 mm i.d., in six parallel horizontal rows connected by glass U-bends | SA=1.2 m², capacity=65 L | 9.4 | $7\times10^{-6}$ mol photon/m²/s | Lab Scale | 35 °C | Two double-jacket heat exchangers in lower degasser and counter-flow cooler (35±1 °C) | 32.5 g/m²/d | [77] |
| Tubular | *Spirulina* sp. | Acrylic glass tubes, 0.13 m diam., 0.004 m thick | SA=100 m²; capacity=100 L/m² | 9.4–9.8 | – | Commercial | 36 °C | Shading with dark plastic sheets; overlapping of tubes; spraying with water | 0.01 g/L/g (air)/m² | [60] |

The growth medium is circulated from a reservoir through the reactor using an airlift device without moving parts, reducing contamination and preventing the cell damage caused by mechanical pumping. Airlift devices also remove excess oxygen, which would otherwise inhibit photosynthesis [54,57,58]

Culture efficiency is highly dependent on optimizing flow and gas exchange, and photobioreactor geometry should also maximize the illumination area. Volumetric productivity decreases as tube diameter increases, while areal productivity increases with volume. For maximum production in a given area, the suggested tube diameter is 0.06 m. The annual areal productivity of *P. tricornutum* using 0.06-m diameter tubes was 35 g/m$^2$/d and 1.5 g/L/d [59].

Tubular photobioreactor can also be designed helically to provide a larger surface area to volume ratio. This design maximizes light penetration, limits contamination, allows for easy temperature control, and provides maximum $CO_2$ transfer in the culture medium. *P. tricornutum* grown in a helical photobioreactor had 1.5 g/L/d biomass productivity at 30 °C [47].

*Spirulina* was cultured in a flat inclined photobioreactor made of flat glass tank with the temperature maintained below 35 °C. The inclined face was set at angles of 30° in the summer and 60° in the winter in the northern hemisphere [60].

The cyanobacterium *Aphanothece microscopica* was cultured in a bubble column photobioreactor, and the effects of photoperiod on biomass productivity and $CO_2$ fixation rates were studied at 35 °C. The duration of light directly impacted biomass productivity, and $CO_2$ fixation rates reduce by 99.69% when deprived of light [61].

Airlift photobioreactors are simple and cost-effective reactors for the mass culture of various types of algae. They are made of acrylic glass, which is inexpensive and easily obtained, and meet the desired criteria for new generation photobioreactors of high light penetration and biomass production, ease of maintenance, and minimal contamination [62]. They have three major parts, a draft tube, an outer tube, and an air duct. They are well suited for cultivating *Chlorella* sp. Volumetric productivity obtained at a superficial velocity of 4 mm/s was 0.21 g/L/d [63].

Ozkan [64] studied the use of performance of an algae biofilm photobioreactor that offers a significant reduction of the energy and water requirements of cultivation. The green alga *B. braunii* was cultivated as a biofilm. The system achieved a direct biomass harvest concentration of 96.4 kg/m$^3$ with a total lipid content 26.8% by dry weight and a productivity of 0.71 g/m$^2$ day, representing a light to biomass energy conversion efficiency of 2.02%. Moreover, it reduced the volume of water required to cultivate a kilogram of algal biomass by 45% and reduced the dewatering energy requirement by 99.7% compared to open ponds.

*Porphyridium purpureum* was cultivated in a bubble column photobioreactor with a diameter of 0.4 m and height of 0.1 m to maximize volumetric productivity. Air was supplied with flow rate of 2.5 L gas per L liquid per hour along with 2% $CO_2$ from below to provide agitation and fixation [65].

Flat plate photobioreactors as shown in Fig. 3 are very effective for biomass cultivation of microalgae. They provide a high surface area to volume ratio for illumination and have easy design features [66]. Biomass productivity of microalgal cultures rapidly increases with mixing rate, which provides an adequate supply of $CO_2$ to the culture while removing excess oxygen and increasing the flashing effect. However, because of higher running costs, high aeration rates are not recommended for large-scale production. *Synechocystis* (Cyanobacteria) was cultivated in vertical flat plate reactors and aeration along with 5–10% $CO_2$ introduced from the bottom of the column to increase biomass productivity [67].

The effects of $CO_2$ aeration rates from 2% to 20% on the biomass productivity of *B. braunii* were investigated in a closed airlift photobioreactor. All strains could grow at all $CO_2$ concentrations

with no obvious inhibition at an aeration rate of 0.02 volume gas per volume liquid per minute. Maximum biomass productivity was 2.31 g/L on the 25th day at a 20% $CO_2$ aeration rate. Moreover, cultivation and harvest could be made more economical by using sodium hypochlorite to sterilize the reactor, by not adjusting the pH, and by using flocculation to harvest the algae [32].

Studies have been done on the production of lipids using *C. vulgaris* and wastewater in a batch or semi-continuous manner in a column aeration photobioreactor. This is a promising means for lipid production. Maximum lipid production was 42% with biomass productivity of 147 mg/L/d using a semi-continuous process with daily replacement rate of one of the two cultures, making it competitive with petroleum at US $63.97/barrel [68].

A penthouse-roof photobioreactor was used to cultivate *Spirulina*. These reactors comprise both indoor and outdoor units and are most efficient in temperate climate zones. They use collectors to concentrate light and all cultivation parameters (temperature, flow rate and oxygen concentration) can be easily controlled. The reactor in this study had a tilt angle of 40° to provide maximum light. *Spirulina* maximum biomass concentration was 1.2–2.2 g/L and productivity was 0.5 g/L/d in September, with a biomass yield of 32.5 g/m$^2$/d [69].

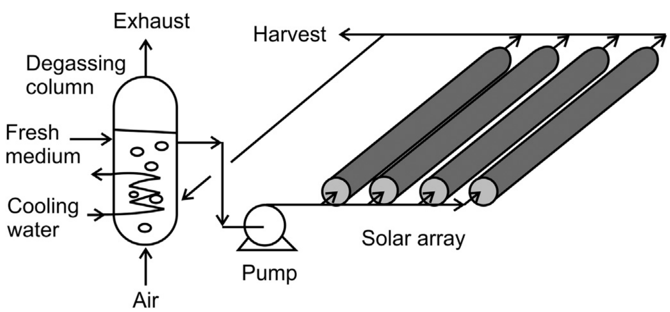



**Fig. 2.** Working of a horizontal tubular photobioreactor.

In Arizona, USA, groundwater provides about 40% of the drinking water, so it is a precious resource. Ten percent of Arizona groundwater exceeds the maximum allowable concentration of nitrates (10 mg/L), making it unfit for drinking. Growing algal cultures using such water could make cultivation more economical and also purify water. To test this potential, *Scenedesmus* (Planta: Chlorophyta) was grown in an outdoor multistage photobioreactor consisting of six flat glass plates arranged in ascending levels (total capacity, 900 L) [73]. The culture was provided with air along with 0.05–1% $CO_2$ through submerged tubing, which also mixed it. The reactor was designed to overflow from one plate to another under gravity, making it very effective at maximizing biomass productivity and also removing nitrates.

*C. vulgaris* was studied in flat plate airlift (FPA) and bubble column photobioreactors provided with 3 cm LPs, 980 E/m$^2$/s of light, and caloric values of 25 kJ/g [71]. The volumetric productivity in the FPA (0.045 g/L/h) was 1.7-fold higher than in the bubble column photobioreactor (0.027 g/L/h), and the photosynthetic efficiency was also higher (4.7% vs. 2.9%). Thus, FPA was considered to be more effective. The advantages and disadvantages of different types of photobioreactors used to cultivate microalgae are listed in Table 3.

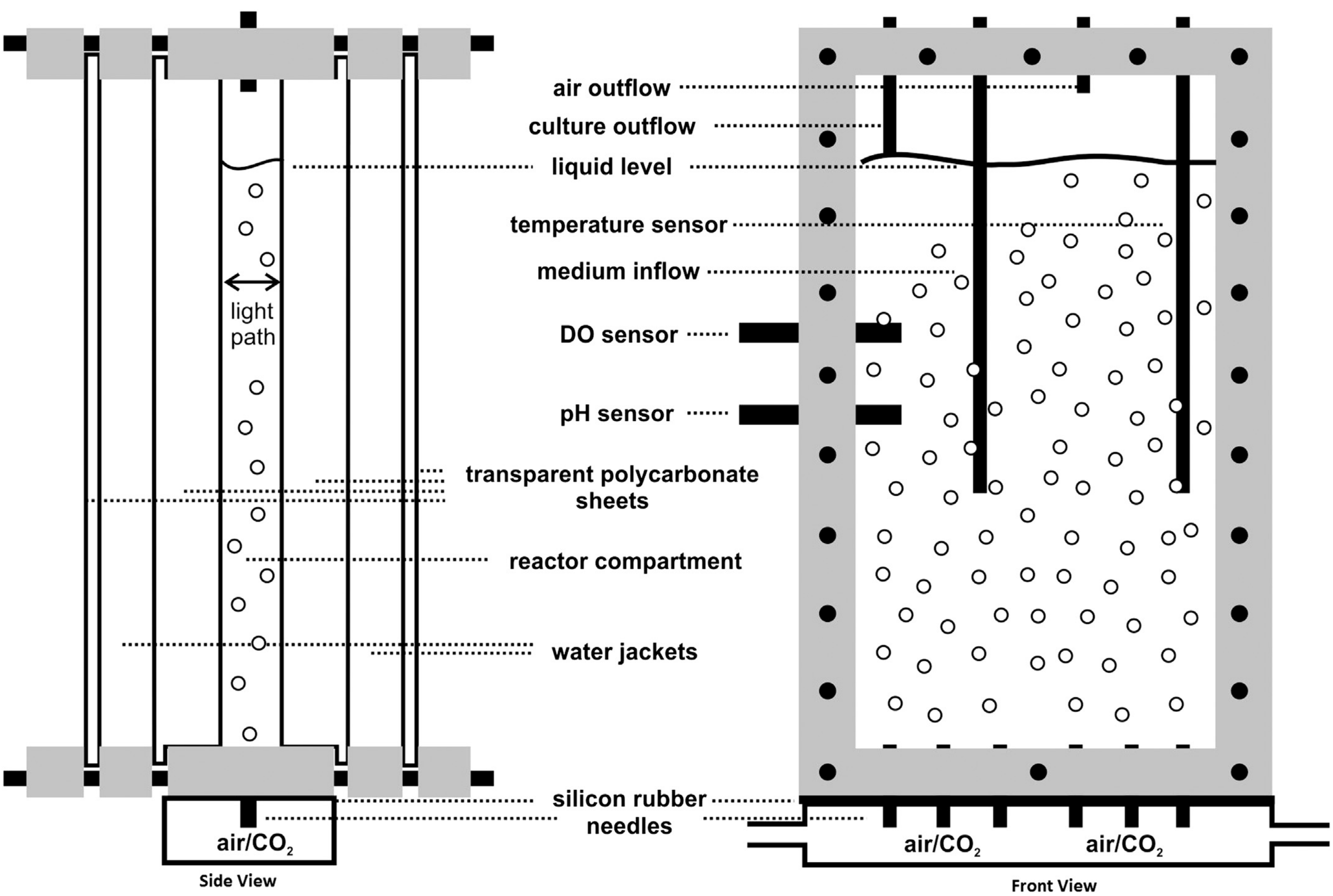



**Fig. 3.** Front and side view of the flat panel photobioreactor.

**Table 3**
Advantages and disadvantages of different photobioreactors used to cultivate microalgae.

| Type of photobioreactor | Advantages | Disadvantages | Reference |
|---|---|---|---|
| Tubular | Very effective light use; excellent temperature control; reasonable scale-up | Fouling with some growth along walls | [78] |
| Vertical | Very high productivities and cell densities | Scale-up requires many compartments and support materials | [79] |
| Flat panel tubular with fresnel coating | Absorbs more oblique light from light source | Difficulty controlling temperature; possible hydrodynamic stress to some algal strains | [80] |
| Helical tubular | High surface area | High heating and illumination costs | [81] |
| Air lift tubular | Capable of large scale production; easy $CO_2$ supply | Complexity; difficult to scale up | [62] |
| Multiple airlifting membrane | Control over overall gas holdup and liquid circulation; production of metabolites | High production cost | [82] |
| Cuboidal | High cell concentration; effective total light incidence | May be dark regions away from the center, depending on reactor depth | [83] |
| Stirred draught tube | Combines stirred tank and plate reactors; constant light conditions | Higher cultivation cost | [84] |
| Stirred tank | Largely uniform mixing; excellent temperature control | Difficult to scale up; hydrodynamic stress on algae | [78] |
| Air lift | Good light use; high temperature control; high mass transfer coefficient | Low hydrodynamic stress on algae; difficult to scale up | [85] |
| Flat plate | Excellent light use and temperature control; high gas transfer coefficient | Difficult to scale up | [67] |
| Bubble column tubular | Scalable; homogeneous culture environment; low cooling requirement; effective light use | Low surface to volume ratio | [85] |
| Modified cascade | Effective light use; high mass transfer coefficient; economical | Increased shear stress by pumps may limit biomass productivity | [86] |

**Table 4**
Methods used to extract lipids and oils from microalgae.

| Product | Extraction method | Species | Solvent | Efficiency/yield (wt%) | Time (min) | *T* (°C) | *P* (MPa) | Reference |
|---|---|---|---|---|---|---|---|---|
| Lipids | Organic solvent | *Chaetoceros muelleri* | 1-Butanol | 94 | 60 | 70 | – | [113] |
| Lipids | Organic solvent | *Chlorococcum* sp. | Isopropanol/hexane | 6.8 | 450 | 25 | – | [90] |
| Lipids | Organic solvent | *Chlorococcum* sp. | Hexane system | 1.5 | 450 | 25 | – | [90] |
| Lipids | Organic solvent | *Phaeodactylum tricornutum* | Ethanol, 5 mL/g dried microalgae | 29 | 1440 | – | – | [114] |
| Lipids | Cold pressing | *Scenedesmus obliquus* | Ethanol | 62.04 ± 2.42 | – | 73–75 | – | [106] |
| Lipids | Bead beater+solvent | *Botryococcus braunii* | Chloroform/methanol | 28.6 | 50 | – | – | [96] |
| Lipids | Bead beater+solvent | *Botryococcus* sp. | Chloroform/methanol | 28.1 | – | – | – | [96] |
| Lipids | Soxhlet | *Scenedesmus obliquus* | Hexane | 40.71 ± 4.46 | – | 63–65 | – | [106] |
| Lipids | Soxhlet | *Botryococcus braunii* | DBU[a]/octanol | 81 | 240 | 60 | – | [115] |
| Lipids | Soxhlet | *Chlorella vulgaris* | Hexane | 1. 77 | 140 | 70 | – | [102] |
| Lipids | Soxhlet | *Chlorococcum* sp. | Hexane system | 3.2 | 330 | – | – | [90] |
| Lipids | Supercritical fluid | *Crypthecodinium cohnii* | $CO_2$, 10 g/min | 9 | 180 | 49.85 | 30 | [116] |
| Lipids | Supercritical fluid | *Chlorococcum* sp. | $CO_2$ | 5.8 | 80 | 60 | 10–50 | [90] |
| Lipids | Subcritical ethanol | *Nannochloropsis* sp. | Ethanol | 90.21 | – | – | – | [117] |
| Lipids | Supercritical fluid | *Nannochloropsis* sp. | $CO_2$ | 25 | | 40 | 55 | [118] |
| Lipids | Supercritical fluid | *Spirulina maxima* | $CO_2$ | 3.1 | | 35 | 60 | [119] |
| Lipids | Supercritical fluid | *Spirulina platensis* | $CO_2$ | 8.6 | 60 | 40 | 40 | [120] |
| Oils | Organic solvent | *Oedogonium* sp. | Hexane+ether | 9.20 | 20 | 80 | – | [112] |
| Oils | Bead beater+solvent | *Chlorella vulgaris* | $CO_2$ | 13.30 | – | – | – | [112] |
| Oils | Soxhlet | *Isochrysis galbana* | $CO_2$, 2.0 mL/min | 4–10 | – | 40 | 69 | [121] |
| Oils | Soxhlet | *Isochrysis galbana* | $CO_2$/ethanol | 5–11 | – | 50 | 6.89 | [121] |
| Oils | Supercritical fluid | *Spirulina platensis* | $CO_2$ | 90 | 15 | 55 | 70 | [112] |
| Oils | Supercritical fluid | *Tetraselmis chui* | DCM[b]/MeOH in a ratio of 9:1 | 15 | – | 100 | 10.34 | [112] |

[a] DBU, 1,8-diazabicyclo-[5.4.0]-undec-7-ene.
[b] DCM, Dichloromethane.

## 4. Extraction

To extract lipids from microalgae, the algae must first be harvested from photobioreactors or open pond cultures and concentrated by filtration, centrifugation, flocculation, or agglomeration to remove the water [87]. Dewatered algae is then dried, milled into a fine powder, and pretreated by bead milling, microwaving, chemical lysis, or high-pressure homogenization to increase the mass transfer of lipids during extraction. Pretreatment greatly improves the extraction efficiency by disrupting the cellular structure, releasing lipids into the solvent mixture, and enhancing overall yield. Oil expellers and presses along with hexane solvent extraction can increase lipid yield up to 75 wt% [88,89].

Lipids can be extracted from the dried algal biomass using different chemical and physical means. Chemical extraction uses organic solvents like hexane or methanol. Other techniques include expeller presses, electromagnetic methods, direct liquefaction, Soxhlet extraction, supercritical fluids ($CO_2$), ultrasonic waves, and microwave-assisted organic solvent extraction [90]. Several methods are summarized below and in Table 4.

After lipid extraction, the remaining constituents (solvent, water, cell debris, and unextracted lipids) are sent to a solid–liquid separation system to remove cell debris [91]. In organic solvent extraction, water and solvent are removed using liquid–liquid separation methods, such as evaporation, vacuum distillation, or solvent adsorption. In supercritical fluid extraction, the mixture is

pressure decomposed, converting the solvent and residual water into gases and precipitating the lipids. Extracted lipids are then transesterified into biodiesel [90].

Technology for the production of biodiesel from microalgae must have high specificity for lipids to minimize contaminants such as carbohydrates and proteins [92]. Purification technology should also favor the production of acylglycerols over other lipids, such as ketones, chlorophylls, sterols, polar lipids, and carotenes, that are not readily converted to biodiesel [93]. Additionally, the technology should have low operating and capital costs, require little energy and time, be safe, and should not react with lipids [89].

### 4.1. Organic solvent extraction

Lipid–solvent systems are governed by the principle that like dissolves like, so lipids are extracted using non-polar organic solvents like chloroform or hexane [94]. The extraction can be divided into five steps. (1) Microalgae are exposed to the solvents, which penetrate the cell membrane and enter the cytoplasm. (2) The solvents interact with neutral lipids via Van der Waal's forces to form a solvent–lipid complex. (3) This complex diffuses across the cell membrane, such that neutral lipids enter the organic phase while water and solvent–contaminant complexes (with carbohydrates or proteins) enter the aqueous phase. (4) The organic phase is then separated, and (5) crude lipids are transesterified to produce biodiesel [7,88,95].

To increase lipid yield, two or more organic solvents can be used simultaneously in polar/non-polar combinations, e.g., methanol/chloroform or hexane/isopropanol. One study found that using isopropanol as a co-solvent increased lipid yield from *Chlorococcum* sp. up to 300% more than using pure hexane; yields were 0.068 and 0.015 g lipid/g algal biomass, respectively [90]. In another study, bead-beaten *B. braunii* was exposed to five different organic solvents; chloroform/methanol yielded the highest lipid content (0.29 g/g algal biomass) [96].

### 4.2. Soxhlet solvent extraction

Organic solvent extraction is usually carried out in a non-continuous batch process, which limits lipid mass transfer equilibrium [97]. To solve this problem, a continuous solvent extraction process is required, which requires large amounts of solvent [98]. Soxhlet solvent extraction continuously evaporates and condenses the solvent, avoiding the lipid mass transfer limitation and reducing solvent consumption [99]. The Soxhlet apparatus comprises a round-bottom flask holding solvent, a Soxhlet extractor containing algal biomass, and a condenser. Heated solvent enters the condenser, which channels it into the extractor. A thimble filter can be used to prevent algal biomass from being carried out of the extractor with the solvent [100]. When the organic solvent reaches its maximum volume in the extractor, it is siphoned into the flask, where it is again heated and evaporated, leaving crude lipids behind [99].

Soxhlet lipid extraction is more effective than batch extraction. Yields from *Chlorococcum* sp. were 0.015 g lipid/g dried microalgal biomass using Soxhlet extraction and 0.057 g lipid/g biomass using a batch process [90]. However, continuous distillation is energy-consuming.

### 4.3. Ultrasonic-assisted organic solvent extraction

Ultrasonic waves can be used to increase lipid yield as compared with other extraction methods. Ultrasonic methods can also extract other biochemical compounds, such as carotenoids and chlorophyll. In the reactor, ultrasonic waves create solvent bubbles that explode and rupture the cell walls, forcing constituents out of the cells into the solvent mixture [91,101]. An oil yield of 1.77 wt% was obtained from *C. vulgaris* using ultrasonic-assisted solvent extraction in 2.33 h, while Soxhlet extraction yielded 1.58 wt% oil after 18 h [102]. Thus, a higher yield was obtained in less time using ultrasonic waves versus Soxhlet extraction.

To avoid filtration and other chemical extraction techniques, microalgal suspensions can also be placed in an electromagnetic field and the pH varied using $CO_2$; this process destroys the cell wall and allows the oils to float to the surface. Lipids can be extracted from live algal cultures using mesoporous nanoparticles [103–105].

### 4.4. Microwave-assisted organic solvent extraction

Microwaves combined with organic solvent can also be used to extract lipids from microalgae. The process uses electromagnetic radiation within a specific frequency range to heat the cells and increase the internal pressure. The cells rupture, forcing their constituents out. The rapid explosion rapidly diffuses the lipids into the organic solvent. Microwave-assisted hexane extraction yielded more lipids than other conventional heating methods and was more rapid [106]. The process is economical and environmentally friendly.

The microalga *Scenedesmus obliquus* was heated to 80–95 °C using microwaves (1.2 kW, 2450 MHz frequency) for 20–30 min. Maximum oil yield obtained was 76–77 wt% at 95 °C with a holding time of 30 min, whereas solvent extraction yielded 52 wt% oil [106,107].

### 4.5. Direct liquefaction

Oils can be directly obtained from dried or wet microalgae by liquefaction. Algae have high moisture content (up to 78.4%), requiring a lot of energy to dewater. Liquefaction directly converts the biomass into oil. Maximum oil yield was 25–44.8 wt% at 300–360 °C and 10 MPa. In experiments with high-moisture *B. braunii* treated with or without a catalyst (5% $Na_2CO_3$) at 300 °C, more than 95% of hydrocarbons were recovered [96].

The origin oil company introduced a new method that can extract oil from microalgae at a rate of 5 gallons/min with an efficiency of 94–97 wt% [53] without requiring dewatering, making the process economical for commercial use. The method combines an electromagnetic field to rupture the cells, pH adjustments, and tank settling and gravity clarification [108].

### 4.6. Supercritical ($CO_2$) fluid extraction

Supercritical $CO_2$ (SC-$CO_2$) extraction is a green technology that promises to replace organic solvent extraction. When the temperature and pressure of a fluid increase above its critical point, the fluid behaves as both a liquid and a gas. This method is very efficient for extracting lipids for several reasons. (1) The crude lipid products are solvent free. (2) The solvent rapidly penetrates the algal cells, giving a higher lipid yield. (3) Solvent power is a function of fluid density, which can be tuned by adjusting the temperature and pressure to get neutral lipids (acylglycerols). (4) Supercritical fluids are non-corrosive, non-toxic, non-flammable, and inert. (5) No degumming is required because SC-$CO_2$ does not solubilize polar phospholipids [109–111].

Supercritical organic solvent extractions use a decompressed $CO_2$ flow rate of 400 mL/min, with a holding time of 4.9–14.1 min depending upon fluid density. The temperature is varied from 60 to 80 °C and the pressure from 10 to 50 MPa for 80–120 min. Under optimum conditions, i.e., 30 MPa and 49.85 °C, almost 50 wt% of the oil is extracted from the dried algal biomass after 2 h [112].

Lipid yields using SC-$CO_2$ increase with decreasing temperature and increasing pressure and are much higher in less time than with ordinary solvent extraction. A yield of 0.058 g lipids/g microalgae was obtained from *Chlorococcum* sp. using SC-$CO_2$

with a residence time of 80 min, while Soxhlet solvent extraction yielded 0.032 g lipids/g microalgae after 5.5 h [90]. In one study, 90% of oils were recovered from *Spirulina platensis* in less than 15 min at 70 MPa and 55 °C using SC-$CO_2$, while it took 6 h to extract the same amount by Soxhlet hexane extraction [65].

## 5. Microalgae biofuel products

### 5.1. Bio-hydrogen

The need for energy, global warming, and pollution combined to make research on alternative energy sources vital. Hydrogen has excellent potential to provide energy while alleviating global warming and pollution concerns [122]. Renewable biohydrogen production is of increasing interest as fossil fuel supplies are being depleted [123]. A number of methods can generate renewable hydrogen, such as biomass gasification, electrolysis, and photovoltaic generation, which all produce hydrogen for less than US $20/GJ, which is quite reasonable [124,125].

Hydrogen has the highest energy content per unit weight (142 kJ/g or 16,000 BTU/lb) of the known fuels, although it requires special handling because little energy is needed for ignition and it leaks easily [125]. Hydrogen gas has excellent potential as a renewable energy source because it produces only water when combusted, unlike the carbon pollution of fossil fuels. The only carbon released by hydrogen gas is derived from $CO_2$-fixation and from microbial fermentation [126]. Thus, biohydrogen can be considered carbon-free. Hydrogen is also very good for internal combustion engines and maintains long-term engine efficiency [127,128]

Most hydrogen is used in the fertilizer (≈50%) and petroleum (≈30%) industries. Sales have increased by 6% per year in the last 5 years, indicating the increased production in refineries. Hydrogen produced today comes from natural gas (40%), heavy hydrocarbons like naphtha (30%), coal (18%), and electrolysis (4%). Biological hydrogen has become a viable source given the current energy demand and environmental issues. The main objective is now to improve hydrogen yield to make it more economically viable [129]. A number of methods are currently being used to produce biohydrogen, for example, hybrid bioreactors, electrochemical-assisted bioreactors, and metabolic and genetic engineering techniques [127].

The main problem for commercializing biohydrogen production is the low yield and rate of production. Using cheaper raw materials, efficient production techniques, and pilot tests of photofermentation plants should make biohydrogen a commercially viable source of energy in the near future [130].

#### 5.1.1. Biophotolysis

Blue-green and green algae produce biological hydrogen by photolyzing water using solar energy and hydrogenase and/or nitrogenase enzymes. Microalgae use solar energy to transfer electrons to NADPH and ferredoxin, which in turn generates hydrogen. This process was studied by Gaffron and Rubin [131]. To offer an economically-competitive source of hydrogen, these organisms must achieve at least 10% solar conversion efficiency; the green alga *Chlamydomonas reinhardtii* achieved the maximum theoretical light-conversion efficiency of 22% under controlled laboratory conditions, as summarized in Table 5 [125]. Photolysis can be further classified into several subcategories, as discussed below.

#### 5.1.2. Direct photolysis

Direct photolysis involves splitting water into hydrogen and oxygen using sunlight energy, as follows:

$$2H_2O + \text{light energy} \rightarrow 2H_2 + O_2$$

*C. reinhardtii* under anaerobic conditions is widely used to produce hydrogen. These hydrogen generated ions are then used for the production of hydrogen in the medium of ferredoxin which are catalyzed by hydrogenase enzyme present in the algal cells. Hydrogen is produced in two steps. First, photosystem II absorbs light and generates electrons that are transferred to ferredoxin using light energy absorbed by the photosystem. Hydrogenase

**Table 5**
Hydrogen production from algae.

| Species | Process | Catalyst | Yield | Time (h) | Reference |
|---|---|---|---|---|---|
| *Chlamydomonas reinhardtii* | Biophotolysis | Hydrogenase | 40 mol% more with blockage of DCMU[a] | 96 | [125] |
| *Chlamydomonas reinhardtii* | Direct biophotolysis | – | – | – | [138] |
| *Anabaena variabilis* (blue algae) | Indirect biophotolysis | Hydrogenase and nitrogenase | – | – | [122] |
| *Chlamydomonas reinhardtii* | Direct biophotolysis | Hydrogenase enzyme | – | – | [122] |
| Blue-green algae | Biophotolysis | Hydrogenase and nitrogenase | 0–5 mol% | – | [141] |
| Green algae | Direct biophotolysis | In vitro chloroplast–ferredoxin–hydrogenase system | – | – | [141] |
| *Rhodopseudomonas sphaeroides* | Biophotolysis | Pigment–protein antennae complexes | 2.2 mol% increase with two-compartment flat plate reactor | – | [135] |
| *Chlamydomonas reinhardtii* | Direct biophotolysis | Bacterial hydrogenases/ferredoxin | 10 mol% | 0.25 | [142] |
| *Chlamydomonas reinhardtii* | Dark fermentation | Hydrogenase and nitrogenase | 20 mol% | – | [142] |
| Green algae | Biophotolysis | Bacterial hydrogenases/ferredoxin | 10–13 mol% | – | [143] |
| *Chlamydomonas reinhardtii* | Direct biophotolysis | Hydrogenase enzyme | – | – | [136] |
| *Anabaena variabilis* | Indirect biophotolysis | Bacterial hydrogenases/ferredoxin | – | – | [136] |
| *Scenedesmus obliquus* | Biophotolysis | Hydrogenase enzyme | 15–20 mol% | – | [144] |
| *Chlamydomonas reinhardtii* | Direct biophotolysis | Fe hydrogenase | 10–20 mol% more with methylamine hydrochloride | – | [137] |
| *Chlamydomonas reinhardtii* | Biophotolysis | Dilution S-deprivation | 23.6 mL/h | 182 | [145] |
| *Chlamydomonas reinhardtii* | Biophotolysis | Nutrient control of S-deprivation | 112.7 mL/h | 210 | [145] |

[a] DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

then accepts the electron from ferredoxin to generate hydrogen

$$H_2O \rightarrow \text{Photo system II} \rightarrow \text{Photo system I} \rightarrow \text{Fd Hydrogenase} \rightarrow H_2$$
$$\downarrow$$
$$O_2$$

Hydrogenase activity is also found in *S. obliquus*, *Chlorococcum littorale*, *Platymonas subcordiformis*, and *C. fusca* but not in *D. salina* or *C. vulgaris* [122,132,133].

*C. reinhardtii* in the presence of 2–7 mM methylamine hydrochloride produces 10–20% more hydrogen (cf. Table 5).

#### 5.1.3. Indirect photolysis

Some cyanobacteria, such as non-marine *Anabaena* sp., marine *Calothrix* sp., *Oscillatoria* sp., *Synechococcus* sp., and *Gloeobacter* sp., can fix nitrogen and produce hydrogen via both hydrogenase and nitrogenase [122,126,134]. Indirect biophotolysis is well suited for nitrogenase-based systems. The reaction is

$$12H_2O + 6CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + O_2$$

$$C_6H_{12}O_6 + 12H_2O + \text{light energy} \rightarrow 12H_2 + 6CO_2$$

However, hydrogen production is 1000-fold lower in nitrogenase-based systems than in reversible hydrogenase-based ones.

When *Rhodopseudomonas sphaeroides* [135] was cultured in a flat plate reactor with two compartments, it showed a 1.4-fold increase in hydrogen conversion efficiency (to 2.2%) relative to wild type with both compartments, as shown in Table 5. *Anabaena* is now being considered for hydrogen production. It has a solar conversion efficiency of about 1–2%, with a maximum efficiency of 16% [136].

Nitrogenase is inhibited by oxygen, so oxygen must be excluded from these systems. Also, $CO_2$ concentrations between 4 and 18% w/v are optimal, resulting in higher cell densities and more hydrogen production [137,138]. Researchers believed that one-third of the hydrogen stored in carbohydrates can be recovered by dark fermentation, for a maximum yield of 20% [135,139]. Current photolysis yields under laboratory conditions are 15–20% [140]. However, hydrogen is presently produced mostly by dark or photofermentation. Photosynthetic hydrogen production is best for converting solar energy into hydrogen, so the current focus of research is to increase light-use efficiency and to design better reactors for hydrogen production [132].

### 5.2. Biodiesel

#### 5.2.1. Introduction

Rudolph diesel first produced methyl esters (diesel) from crops in 1900 [146], and biodiesel has since received much attention as a renewable, biodegradable, and nontoxic source of diesel [147,148]. The United Kingdom consumes nearly 25 billion L of diesel annually; to produce this much biodiesel using oil seeds would require more than half of the land in the UK. In recent years, microalgae have been increasingly considered promising sources of biodiesel because of their high reproduction rates and lipid contents (50–70%); the lipids are transesterified into methyl esters. Algal biodiesel can contain 39–41 MJ/kg caloric value, close to that of petrodiesel (46 MJ/kg) [149]. A recent survey indicated that to fulfill

**Table 6**
Advantages and disadvantages of biofuel derived from microalgae.

| Advantages | Disadvantages |
|---|---|
| High growth rate | Low biomass concentration |
| More cost effective farming | Difficult to harvest due to microscopic size of most planktonic microalgae |
| Less water demand than land crops | Algae can grow on brackish water from saline aquifers or in sea water. While this may solve some of the water availability problems, it will result in other undesirable side effects: salt precipitation on the bioreactor walls; precipitates on pumps and valves leading to reduced lifecycle; presence of salts in the final biomass, which will likely have to be purged with steam. |
| High-efficiency $CO_2$ mitigation | There is a need to develop techniques for growing a single species, reducing evaporation losses and increasing the utilization of $CO_2$. |
| Algae biofuel contains no sulfur | Drying and extraction is difficult. In dry extraction (drying the algae by using the sun or artificially), they receive a much lower yield. When using artificial dryers (such as using electricity) it takes more energy to extract than the energy you can get from the yield. |
| Algae produce nontoxic and highly biodegradable biofuels. | Natural algal stands are not favoured probably due to their low productivity for target organisms. Most of microalgae species are unadapted to local climates and outdoor cultivation. |
| Growing algae do not require the use of herbicides or pesticides. | Drying and extraction is difficult. In dry extraction (drying the algae by using the sun or artificially), they receive a much lower yield. When using artificial dryers (such as using electricity) it takes more energy to extract than the energy you can get from the yield. |
| High levels of polyunsaturates in algae biodiesel is suitable for cold weather climates | Biodiesel performs poorly compared to its mainstream alternative. |
| A high per-acre yield (7–31 times greater than the next best crop—palm oil) | Produces unstable biodiesel with many polyunsaturated |
| Easy to provide optimal nutrient levels due to the well-mixed aqueous environment as compared to soil | Limited genomic data for algal species |
| Continuous production avoids establishment periods of conventional plants | Large scale extraction procedures for microalgal lipids are complex and still in development stage. |
| Ability to adjust harvest rates to keep culture densities at optimal levels at all times. Especially with the continuous culture systems, such as raceway ponds and bioreactors, harvesting efforts can be controlled to match productivity. | Microalgae grown in open pond systems are prone to contamination. |
| Algae oil extracts can be used as livestock feed and even processed into ethanol | There exist few commercial cultivating "farms", so there is a lack of data on large-scale cultivation. |
| Capability of performing the photobiological production of biohydrogen. | Higher capital costs |
| Algae-based fuel properties allow use in jet fuels. | Large-scale production could present many other drawbacks compared to those found in laboratory experiments. |

50% of the US fuel needs using algae would require only 1–3% of the total US cropping area, while using palm oil would require 24% of that area [54]. Table 6 lists the advantages and disadvantages of biofuel derived from microalgae [42,54,147,149,150].

There are about 300,000 species of algae, some of which, like *C. reinhardtii*, *D. salina*, and various *Chlorella* species, can contain 60% lipids. For algae, concerns are whether to use closed or open photobioreactors, measures to prevent contamination, and how to supply nutrients and $CO_2$ to the cultures. Biodiesel can be extracted from algae using expellers, hexane extraction, or supercritical $CO_2$ extract, with up to 70–75 wt% oil [151].

Biodiesel does not require engine modifications and also reduces CO (by 50%) and $CO_2$ (by 78%) emissions [54]. Algal fuel currently costs about US $52–91/barrel (based on the inflation of 2008) [152].

A main problem of algal biofuels is their high viscosity, which can be 10–20 times more than that of no. 2 diesel fuel. Such high viscosity fuels are difficult to combust and leave deposits on the fuel injector of diesel engines. Therefore, biodiesel is usually blended with conventional diesel. Four techniques can address this problem: pyrolysis, micro-emulsification, dilution, and transesterification [105,153]. Transesterification offers the most promise for lowering viscosity.

### 5.2.2. Transesterification

Transesterification is the chemical conversion of triglycerides into methyl esters using a solvent and catalyst. The reaction converts three moles of alcohol and one mole of triglyceride into one mole of glycerol and three moles of methyl esters, although excess methanol is used to promote biodiesel formation [148,154], giving methyl ester yields greater than 98% w/w [54]. Catalysts include acids (e.g., sulfuric acid), bases (e.g., sodium hydroxide, potassium hydroxide), supercritical fluids, and enzymes such as lipase. Experimentally, base-catalyzed reactions were 4000 times faster than acid-catalyzed ones. Commercially, alkoxides of sodium and potassium are used at 1% per weight of oil formed because of their better catalytic activity compared with simple alcohols. Base-catalyzed reactions are optimized at 60 °C under atmospheric pressure for 90 min. At higher temperatures and pressures, the reaction proceeds faster but is more expensive. The reaction yields two layers, because the excess methanol is insoluble in oil. The biodiesel is then separated from contaminants, e.g., glycerol and solids in a flask separator [155]. Biodiesel may contain free fatty acids which can cause saponification, so after separation the oil is washed with 5% water to prevent yield loss. Transesterification consumes 4.3 MJ/L of biodiesel [148].

The optimum conditions for acidic transesterification are 100% catalyst (e.g., sulfuric acid), a methanol-to-oil ratio of 56:1, and a temperature of 30 °C. The specific gravity of oil reduces from 0.912 to 0.8637 in 4 h [148].

Transesterification by using lipases at low temperatures yields more biodiesel than other transesterification methods. The amount of lipase affects the reaction rate; using 75% immobilized lipase with 10% water gives a 98.15% conversion in 12 h. Unfortunately, lipases are too expensive for commercial production of biodiesel and are deactivated by impurities [156–158].

*Nannochloropsis oculata* was transesterified in the presence of CaO and $Al_2O_3$ catalysts at 50 °C to give an oil conversion of 97.5% [159] (Table 7). In another experiment, algae were transesterified in the presence of zirconia, titania, and alumina catalysts at 350–400 °C and 2500 psi (17.23 MPa), giving an oil conversion of 90.2% [160]. *C. protothecoides* was transesterified in the presence of 75% lipase (from *Candida* sp.) and methanol at 38 °C, with an oil conversion of 98.15% after 12 h [161]. Biodiesel produced from various microalgae species is listed in Table 7.

### 5.2.3. Transesterification via liquefaction

Liquefaction is a process by which wet algal mass is decomposed into liquid fuel in an autoclave at moderate temperatures (300–350 °C) and pressures (5–20 MPa). Fresh algae (thalli) are first autoclaved for 1 h in a nitrogen atmosphere at 3 MPa to prevent water evaporation. Water heated to subcritical conditions decomposes biomass to smaller high-energy molecules. The autoclave is then cooled, the gas fraction is transferred to a cylinder, and the reaction mixture recovered is treated with dichloromethane to form two phases. The solvent is evaporated from the organic mixture to yield an amber-colored oil.

Liquefaction requires less input energy (300–350 °C) than either pyrolysis (500 °C) or gasification (2000 °C). Liquefaction can also be used for algae with high moisture contents (>80–90 wt%), but reactors are expensive and complex. Supercritical $CO_2$ extraction and liquefaction differ in that the former extracts more long-chain compounds and polysaturates, while liquefaction gives more oils. Quantitatively, liquefaction seems more effective than supercritical $CO_2$ extraction [147].

*B. braunii* was thermochemically liquefied at 300 °C and 10 MPa in the presence of sodium carbonate to yield 57–64 wt% oil. The caloric value was 45.9 MJ/kg, very close to that of petrodiesel [162]. Similarly, *Dunaliella tertiolecta* with 78.4 wt% moisture was liquefied at 340 °C and 10 MPa in a hydrogen environment for 60 min to yield 34.9–37% oil of 34.9–36 MJ/kg [163].

### 5.2.4. Supercritical methanol transesterification

Supercritical methanol transesterification is now being tested for the production of biodiesel. The process is carried out in 100 mL cylinders containing algae, to which methanol is

**Table 7**
Biodiesel production from microalgae.

| Species | Method | Catalyst | *T* (°C) | *P* (MPa) | Time (min) | Yield (wt%) | Reference |
|---|---|---|---|---|---|---|---|
| *Chlorella protothecoides* | Transesterification | $H_2SO_4$ | 30 | – | 240 | 80 | [30] |
| *Botryococcus braunii* | Liquefaction | Sodium carbonate | 300 | – | 120 | | [162] |
| *Dunaliella tertiolecta* | Liquefaction | – | 340 | – | 60 | 37 | [163] |
| *Nannochloropsis oculata* | Transesterification | $CaO/Al_2O_3$ catalyst | 50 | – | 240 | 97.5 | [159] |
| Algae oil | Transesterification | Zirconia, titania and alumina | 300–450 | 17.23 | – | 90.20 | [160] |
| *Chlorella protothecoides* | Transesterification | 75% *Candida* sp. lipase | 38 | – | 720 | 98.15 | [161] |
| *Chlorella protothecoides* | Transesterification | 30% Immobilized lipase | 38 | – | 720 | 98.15 | [161] |
| *Chlorella minutissima* | Transesterification | Sodium methylate | 110 | – | 300 | 82 | [167] |
| | Transesterification | NaOH | 60 | | 120 | 93 | [168] |
| *Chlorella emersonii* | Transesterification | KOH | 90 | | 90 | 88 | [169] |
| *Schizochytrium sp* *Neochloris oleoabundans* | In Situ Transesterification | | 70 | | 120 | 30 | [170] |

**Table 8**
Biohydrocarbon production from microalgae.

| Product | Species | Process | Catalyst/raw material | Yield/conversion | *T* (°C) | *P* (MPa) | HHV MJ/kg | Reference |
|---|---|---|---|---|---|---|---|---|
| Bio-crude | *Nannochloropsis*/ algae species | Hydrothermal conversion | – | 30 wt% | 300 | 8.10 | – | [176] |
| C20–C30 | *Dunaliella parva* | Hydrothermal conversion | – | 15.70 wt% | 350 | – | 36.7 | [177] |
| $CH_4$/alkanes | *Emiliania huxleyi* | Pyrolysis | – | 72 wt% methane relative scale | 400 | – | – | [181] |
| Bio-crude | *Botryococcus braunii* | Hydrocracking | – | 40 wt% (67% jet, 15% diesel) | – | – | – | [196] |
| Alkanes (C15 – C18) | Microalgae oil | Hydrogenation – decarbonylation | Zeolite-supported Ni catalysts/$ZrO_2$ | 75 wt% | 270 | 4 | – | [196] |
| Bio-crude | *Dunaliella tertiolecta* | Direct liquefaction | – | 37 wt% | 300 | 10 | 36 | [163] |
| Bio-crude | *Botryococcus braunii* | Direct liquefaction | 5%sodium carbonate | 64% dry wt basis of oil | 300 | – | 30–35 | [197] |
| CH4/Alkanes | *Nannochloropsis* sp. | Liquefaction in water | Pd/C-catalyzed | 57 wt% | 350° | – | 38 | [187] |
| Bio-crude | *Enteromorpha prolifera* | Hydrothermal liquefaction | $Na_2CO_3$ | 23.0 wt%. | 220–320 | – | 28–30 | [198] |
| Alkanes | Algae species | Hydro treated | 10 wt% Ni/HB with Si/Al ratio of 75 | 78 wt% (60 wt% yield of C18 octadecane, propane (3.6 wt%) and methane (0.6 wt%)) | 260 | 4 | – | [179] |
| Liquid fuel | *Chlorella* | Catalytic pyrolysis | $Na_2CO_3$ | 40 wt% | 400 | – | 21.2 | [180] |
| Bio-crude | *Chlorella vulgaris* | Catalytic pyrolysis | H+ZSM-5 (1:9) catalyst | 52.7 wt% | 500 | – | 18.6 | [180] |
| Bio-crude/ light fraction | *Spirulina platensis* | Thermochemical liquefaction | – | 39.9 wt%/50–63 wt% light biocrude | 350 | | 34.7–39.9 | [188] |
| Bio-crude | *Dunaliella salina* | Hydrothermal liquefaction | Ni/REHY catalyst | 72.00 wt% | 200 | 2.0 | 30.11 | [189] |
| Ethanol | *Chlorella* sp. | Fermentation | Yeast *Saccharomyces cerevisiae* | 65 wt% | – | – | 22 | [175] |
| Bio-crude | *Chlorella vulgaris/ Spirulina* | Hydrothermal liquefaction | Acetic acid | 70–75 wt% (CARBON CONTENT) | 300–350 | – | 23.2/21.2 | [191] |
| Bio-crude | *Botryococcus braunii* | Hydrothermal liquefaction | $Na_2CO_3$ | 57–65 wt% oil | – | – | – | [162] |
| Bio-crude | *Dunaliella tertrolecta* | Hydrothermal liquefaction | $Na_2CO_3$ | 37 wt% | – | – | – | [163] |
| Bio-crude | *Microcystis viridis* | Hydrothermal liquefaction | Sodium carbonate | 33–40 wt% | – | – | – | [190] |
| Bio-crude | *Spirulina* | Catalyzed liquefaction | Sodium carbonate | 20.0 wt% | – | – | – | [191] |
| Bio-crude | *Chlorella* | Catalyzed liquefaction | Sodium carbonate | 27.3 wt% | – | – | – | [191] |
| Bio-crude | *Spirulina* | Catalyzed liquefaction | KOH | 9 wt% | 350 | – | – | [191] |
| Bio-crude | *Chlorella* | Catalyzed Liquefaction | KOH | 13.6 wt%. | 350 | – | – | [191] |
| Bio-crude | *Spirulina* | hydrothermal liquefaction | Acetic acid | 19.5 wt% | 300–350 | – | 21.2 | [191] |
| Bio-crude | *Chlorella vulgaris* | hydrothermal liquefaction | Acetic acid | 15.7 wt% | 300–350 | – | 23.2 | [191] |
| C10–C30 | *Chllorella protothecoides* | pyrolysis | – | 17.50 wt% | 500 | – | 30 | [183] |
| C17–C19 | *Microcystis aeruginosa* | pyrolysis | – | 23.70 wt% | – | – | 29 | [183] |
| Bio-oil | *Spirulina* | Liquefaction in toluene | $Fe(CO)_5$–S | 52.3–66.9 wt% | 350 | 5.0 | 32–33 | [193] |
| Bio-crude | *Spirulina* | Liquefaction in water | – | 78.3 wt% | 350 | – | 26 | [193] |
| Bio-crude | *Chlorella vulgaris* | Hydrothermal liquefaction | $Ni/Al_2O_3$ catalyst | 30 wt% | 350 | 15–20 | 23.2 | [192] |
| Bio-crude | *Nannochloropsis occulta* | Hydrothermal liquefaction | $Ni/Al_2O_3$ catalyst | 18.10 wt% | 350 | 15–20 | 17.9 | [192] |
| Bio-oil | *Dunaliella tertiolecta* | Direct liquefaction | – | 37 wt% | 302 | 10 | 36 | [104] |
| Bio-oil | *Botryococcus braunii* | Catalyzed Liquefaction | Sodium carbonate | 64 wt% basis | 302 | – | – | [149] |
| petroleum oil | *Botryococcus braunii* | Liquefaction | – | 57–64 wt%/( > 95%) recovery | 302 | – | – | [149] |
| Bio-oil | *Dunaliella tertiolecta* | Hydrothermal liquefaction | 5% Sodium carbonate | 25.80 wt% | 360 | – | 30.74 | [199] |
| Bio-crude | *Spirulina* | Thermochemical liquefaction | Ethanol solvent | 22.7 wt% ($C_{18}H_{36}O_2$) | 380 | – | – | [200] |
| Bio-crude | *Spirulina* | Thermochemical liquefaction | Methanol solvent | 35.53 wt% ($C_{18}H_{36}O_2$) | 380 | – | 39.83 | [200] |
| Bio-crude | *Nannochloropsis* sp. | Hydrothermal liquefaction | Sodium carbonate | 42 wt% | 350 | 20 | 17.9 | [194] |
| Bio-crude | *Spirulina* | Hydrothermal liquefaction | – | 32.60 wt% | 300 | 10–12 | 32.0–34.7 | [195] |
| Methanol | *Spirulina* | Gasification | – | 64 wt% | 1000 | – | 16.9 | [172] |

**Table 8** (*continued* )

| Product | Species | Process | Catalyst/raw material | Yield/conversion | *T* (°C) | *P* (MPa) | HHV MJ/kg | Reference |
|---|---|---|---|---|---|---|---|---|
| Bio-oil | *Polytrichum commune* | Pyrolysis | – | 39.10 wt% | 750–775 | – | 17.4 | [184] |
| Bio-oil | *Dicranum scoparium* | Pyrolysis | – | 34.30 wt% | 750–775 | – | 16 | [184] |
| Bio-oil | *Thuidium tamarascinum* | Pyrolysis | – | 33.60 wt% | 750–775 | – | 15.8 | [184] |
| Bio-oil | *Sphagnum palustre* | Pyrolysis | – | 37 wt% | 750–775 | – | 16.6 | [184] |
| Bio-oil | *Drepanocladus revolvens* | Pyrolysis | – | 35.40 wt% | 750–775 | – | 15.9 | [184] |
| Bio-oil | *Cladophora fracta* | Pyrolysis | – | 48.20 wt% | 750–775 | – | 19.8 | [184] |
| Bio-oil | *Chlorella protothecoides* | Pyrolysis | – | 55.30 wt% | 750–775 | – | 23.6 | [184] |
| Bio-ethanol | *Chlorococcum littorale* | Dark Fermentation | – | 450 μmol ethanol $g^{-1}$ | 30 | – | – | [175] |
| Bio-ethanol | *Spirogyra* | Fermentation | – | 14–17 wt% | – | – | – | [201] |

introduced at supercritical conditions (350–400 °C, 10–25 MPa) [164]. The newly-developed McGyan process yields considerably more biodiesel using a fixed-plate metal oxide catalyst instead of supercritical alcohol and does not lose efficiency over an extended period of time [165].

Biodiesel production using supercritical methanol is economical and gives higher yields than other processes. To produce 1 L of biodiesel, transesterification requires 4.3 MJ of energy, while supercritical methanol requires only 3.3 MJ [166].

### 5.3. Biomethanol

Microalgae can also be used to produce biomethanol as a renewable fuel, although bioethanol has received more attention than biomethanol, which is corrosive, toxic, and has a high cold point, which causes engine start problems in cold weather [171]. *Spirulina* is converted into methanol by gasification. Microalgae are first concentrated (1–2% w/v) in settling ponds and centrifuges (2–21% w/v). *Spirulina* oxidizes into gas, the composition of which determines the methanol yield. Temperatures of 850–1000 °C increase the methanol yield; the maximum yield was 0.64 g of methanol per gram of algal biomass at 1008 °C. Oxygen during gasification is supplied by adsorption. Hot steam helps to reform hydrocarbons, and ash, tar and particulates are removed with a scrubber. Carbon dioxide produced by the reaction is removed by absorption using mono-ethanol–amine. *Spirulina* yields a heat value of 40–50 kcal/kg by gasification [172].

### 5.4. Bioethanol

In recent years, ethanol has become an important alternative fuel or fuel supplement. Bioethanol fuel reduces lead, sulfur, CO, and particulate emissions. Ethanol used as a fuel in Brazil reduced carbon emissions by 9.56–106 t, contributing to a reduction of 15% of Brazil's total emissions. Bioethanol can be produced using microalgae as a feedstock for fermentation; bacteria or yeast ferment the carbohydrates, such as glucose and starch, in the microalgae.

The overall reaction for ethanol production from biomass is

$$C_6H_{12}O_6+2ADP+2Pi \rightarrow 2C_2H_5OH+2CO_2+2ATP+2H_2O$$

The process occurs in two steps. First, enzymatic saccharification hydrolyzes large carbohydrates and cell walls to produce starches. Then, yeasts such as *Saccharomyces cerevisiae* are added to convert sugars into ethanol. The ethanol is then purified by distillation.

*C. vulgaris* and *Chlorococcum* sp. are widely used for bioethanol production because of their high starch contents. On average, 3.83 g/L of ethanol is produced from 10 g/L of lipid extracted from algae [173,174]. *C. littorale* produced 450 μmol of ethanol per gram of algae during fermentation at 30 °C [175]. Fermentation of *Spirogyra* yielded 14–17% bioethanol [123]. In one study, *C. vulgaris* achieved a conversion efficiency of about 65%. Fermentation enzymes are more active at 25 °C than at 35 °C, and ethanol production ceases at 45 °C, so lower temperatures are better [175]. See Table 8 for additional examples.

Bioethanol fermentation from microalgae requires less energy than biodiesel production. The undesired $CO_2$ byproduct can be recycled to cultivate additional algae. However, the commercial production of bioethanol from microalgae is still in the research stages [91].

### 5.5. Biohydrocarbons

In an experiment oil obtained by hydrothermal conversion of *Nannochloropsis* water at supercritical conditions was used for hydrolysis. Upon hydrolysis, an organic layer and a solid layer form. The organic layer contains proteins, carbohydrates and fatty acids from the hydrolysis of lipids. Oil is then separated from the aqueous layer via distillation or decanting. *Nannochloropsis* yielded 30% hydrocarbons at 300 °C and 8.16 MPa, while *D. parva* yielded 15.7% at 350 °C [176,177]. Other examples are shown in Table 8.

Alkanes are also produced by hydrogenation and decarbonylation of microalgae. The reaction is catalyzed by synthetic nickel catalyst and $ZrO_2$ support to produce ketone intermediates, which are then hydrogenated to aldehydes in the presence of Ni catalysts. The reaction occurs in either an autoclave or a continuous flow trickle bed reactor, which stabilizes the catalyst. Algae are placed in the reactor at 260 °C with a 40 MPa hydrogen environment. The total liquid yield contained 70–75 wt% of C17 and n-heptadecane [178].

Alkanes in the range of C15–C18 were obtained by hydro treatment of microalgae in the presence of 10 wt% Ni/Hbeta (Si/Al = 180) catalyst in batch mode. Conversion took place at 260 °C in a hydrogen environment at 4 MPa and yielded 78 wt% liquid alkanes (60 wt% C18 octadecane, 3.6 wt% propane, and 0.6 wt% methane). A Ni/HBeta catalyst increases the hydrogenation rate and produces propane and fatty acids from saturated triglycerides [179]. See Table 8 for additional examples.

#### 5.5.1. Pyrolysis

The pyrolysis of microalgae gives up to 40% oil recovery. This process has a high fuel-to-feed ratio and is the most effective way

to convert algal biomass to biofuels to eventually replace non-renewable fossil fuels. Bio-oil production can be considerably improved by using proper catalysts that are effectively preactivated. *Chlorella* when pretreated with $Na_2CO_3$ was less acidic and produced more aromatics with higher caloric value, suggesting that the appropriate catalysts and pretreatment techniques could yield more hydrocarbons than simple pyrolysis alone. After pretreatment, the *Chlorella* was placed in a fixed-bed reactor and heated from above for 30 min to ensure complete conversion of the microalgae. Hydrocarbons were then condensed as they arose from the reactor. The energy conversion efficiency was 40%, with a caloric value of 21.2 MJ/kg [180].

*Emiliania huxleyi*, a marine coccolithophore, is a candidate for hydrocarbon production. Methane was obtained when it was subjected to vacuum pyrolysis at 100–500 °C. The highest hydrocarbon yield obtained was 129 mL at 400 °C, 10 times more than at 300 °C, while fewer liquid saturates and aromatics formed at 400 °C, showing that low-temperature pyrolysis is a direct source of hydrocarbon gases [181].

In an experiment, *C. vulgaris* was pyrolyzed in a fixed-bed reactor in the presence of a 1:9 ZSM-5 catalyst-to-biomass ratio. The yield was 52.7 wt% (Table 6); 25% of the hydrocarbons were alkanes, alkenes, or aromatic compounds. Recent studies show that aromatic yield can be increased to 40 wt% of the bio-oil obtained from pyrolysis by adding appropriate catalysts. Thus, catalysts can both increase the amount of aromatics in bio-oil and also reduce negative outcomes, e.g., oxygen from 30.1 to 19.5 wt%, with an increased caloric value of 32.2 MJ/kg from 24.4 MJ/kg [182].

Recently, fast pyrolysis was performed using *C. protothecoides* and *M. aeruginosa* in a fluid bed reactor. High quality bio-oils were produced when the reactor was heated to 500 °C with an average algal feed rate of 4 g/min, a method that could be used for large-scale commercial production of bio-oils. Bio-oils obtained by fast pyrolysis have reduced oxygen content with higher caloric values of 29 MJ/kg. Bio-oil yields obtained from *C. protothecoides* and *M. aeruginosa* were 17.5 and 23.7 wt% with higher asphaltene and organic content of 35.9 and 29.99 wt% respectively [183].

The bio-oil yield increases considerably as temperature increases from 476.85 to 501.85 °C. The maximum oil yields from *Cladophora fracta* and *C. protothecoides* were 48.2% and 55.3 wt%, respectively (Table 6). Bio-oil yields from *Chlorella* rose from 5.7 to 55.3 wt% as the temperature increased from 202 to 502 °C and decreased to 51.8% at 602 °C [149,184].

### 5.5.2. Direct liquefaction

Bio-hydrocarbons were produced when *D. tertiolecta* and *B. braunii* were subjected to direct liquefaction at 300 °C and 10 MPa; oil yields were 37% and 64% (dry wt basis of oil), respectively (Table 8). Caloric values ranged from 30 to 36 MJ/kg [163,185,186].

Bio-oil was produced from *Nannochloropsis* sp. by direct liquefaction at 350 °C using different catalysts, e.g., Pd/C, Pt/C, Ru/C, Ni/$SiO_2$–$Al_2O_3$, CoMo/γ-$Al_2O_3$, and zeolite, under a hydrogen environment at high pressure. The gaseous products were mainly $CO_2$, $H_2$, and $CH_4$ with minor quantities of $C_2H_2$ and $C_2H_6$. The highest methane yield was obtained using Ru and Ni catalysts. Higher pressures of hydrogen suppress the gaseous fraction during liquefaction. Bio-oil produced by using Ni catalyst had sulfur content below detection levels. Bio-hydrocarbon yield was 35% from non-catalyzed liquefaction reactions, while a Pd/C catalyst in an $H_2$ atmosphere increased yield up to 57%, with lesser amount of nitrogen [187].

During an investigation of the production of bio-oil from *S. platensis*, maximum hydrocarbon yield was 39.9% with a carbon conversion efficiency of 98.3% at 350 °C with a holding time of 60 min. Biocrude obtained above 300 °C had 71–77% elemental carbon and 0.6–11.6% oxygen with a caloric value of 34.7–39.9 MJ/kg (Table 8). Gas chromatography–mass spectroscopy (GC/MS) analysis identified hydrocarbons in the range of C16–C17 [188]. Hydro-liquefaction of *D. salina* was performed in a hydrogen atmosphere with a bifunctional Ni/REHY catalyst at 200 °C and 2 MPa for 60 min. The maximum yield was 72%, with a conversion efficiency of 87.6% (Table 8) [189].

Yang et al. [190] obtained an oil yield of 33–40 wt% from *Microcystis viridis* subjected to liquefaction in the presence of a $Na_2CO_3$ catalyst. Maximum bio-oil yields obtained from *Spirulina* and *Chlorella* using an alkali KOH catalyst at 350 °C were 9 wt% and 13.6 wt%, while yields obtained using acetic acid were 19.5 wt% and 15.7 wt%, respectively [191]. However, when yields were expressed on organic basis, $Na_2CO_3$ produced higher bio-oil yields of 20.0 wt% and 27.3 wt%, correspondingly (Table 8). The different catalysts were ranked by yield in the order $Na_2CO_3 > CH_3COOH >$ KOH > HCOOH [110]. *Spirulina* was liquefied in tetralin at 350 °C for 60 min and yielded 52.3–66.9 wt% oil in the presence of Fe$(CO)_5$–S catalyst in a hydrogen environment at 5 MPa (Table 8). Liquefaction in water gave an oil yield of 78.3 wt% at 350 °C in the presence of nitrogen. GC/MS analysis showed that liquefaction in toluene resulted in higher carbon content with little oxygen and a caloric value of 32–33 MJ/kg [191].

Hydrothermal liquefaction of *C. vulgaris* and *N. occulta* gave maximum oil yields of 30 wt% and 18.1 wt%, respectively, in the presence of a Ni/$Al_2O_3$ catalyst at 350 °C and 15–20 MPa (Table 6) [113]. Hydrothermal liquefaction converts algal biomass to bio-oil thermochemically. Recent work investigated the hydrothermal conversion of *D. tertiolecta* at different temperatures and using various catalysts. A maximum oil yield of 25.8% was obtained at 360 °C with a holding time of 50 min using a 5% $Na_2CO_3$ catalyst (Table 8). The bio-oil obtained was a mixture of ketones, aldehydes, methyl esters, and fatty acids having a caloric value of 30.74 MJ/kg [192].

The organic solvent can greatly impact the bio-oil product in thermochemical liquefaction. In one study, methanol, ethanol, and 1,4-dioxane were tested with *Spirulina*. The bio-oil produced using methanol contained more carbon and hydrogen compounds with little oxygen content and a caloric value of 39.83 MJ/kg. The major component obtained using methanol and ethanol was hexadecanoic acid methyl ester (35.53% and 26.27%, respectively; Table 8), while 1,4-dioxane favored the formation of $C_{16}H_{31}N$ (22.7%) [193]

Thermochemical liquefaction is a process by which algal biomass is converted into bio-oil using extremely hot (300–340 °C), high-pressure (up to 20 MPa) water with or without a catalyst. A maximum oil yield of 33 wt% (organic basis) was obtained when *M. viridis* was subjected to liquefaction in the presence of 5% $Na_2CO_3$ at 340 °C for 60 min (Table 8). The bio-oil composition was 62% carbon, 28% hydrogen, 8% $N_2$, and 2% sulfur. Liquefied oil mainly contained alkanes in the range of C17–C18 [190]. Bio-oil yields obtained by hydrothermal liquefaction of *Nannochloropsis* sp. and *Spirulina* in the presence of 5% $Na_2CO_3$ were 42% and 32.60%, respectively, at 300–350 °C and 10–12 MPa (Table 8) [194,195].

## 6. Production costs and life cycle assessment of algae derived fuels

Liquid fuels production from microalgae is proven technically, but is still expensive compared to petroleum fuels. Algae fuel is economically viable only in a scenario with crude petroleum selling for ≥$100 per barrel [19,202]. Producing microalgal biomass is generally more expensive than growing crops. Photosynthetic growth requires light, carbon dioxide, water and inorganic

salts. Temperature must remain generally within 19–23 °C. To minimize expense, biodiesel production must rely on freely available sunlight, despite daily and seasonal variations in light levels [54]. Microalgae production in closed photobioreactors (PBRs) is highly expensive. Closed systems are much more expensive than ponds. However, the closed systems require much less light and agricultural land to grow the algae. High oil species of microalgae cultured in growth optimized conditions of PBRs have the potential to yield 19,000–57,000 L of microalgal oil per acre per year. The yield of oil from algae is over 200 times the yield from the best-performing plant/vegetable oils [54]. According to Singh et al. [21] more thrust is being made to cultivate algae in closed systems or using photobioreactors i.e. 52% of the world wide technologies being used for algae biofuel production companies, 25% from open ponds and 22% from natural settings. Recovery of oil from microalgal biomass and conversion of oil to biodiesel are not affected by whether the biomass is produced in raceways or photobioreactors. Hence, the cost of producing the biomass is the only relevant factor for a comparative assessment of photobioreactors and raceways for producing microalgal biodiesel. If the annual biomass production capacity is increased to 10,000 t, the cost of production per kilogram reduces to roughly $0.47 and $0.60 for photobioreactors and raceways, respectively, because of economy of scale. Assuming that the biomass contains 30% oil by weight, the cost of biomass for providing a liter of oil would be something like $1.40 and $1.81 for photobioreactors and raceways, respectively [54].

There have been many attempts to estimate this for large scale micro-algae biofuels production using life cycle assessment (LCA) methods to describe and quantify inputs and emissions from the production process. Attempts have been hampered, however, by the fact that no industrial scale process designed specifically for biofuel production yet exists [44]. Slade [44] reviewed the seven recent LCA studies done by Kadam [203], Jorquera [204], Campbell [205], Sander [206], Stephenson [207], Lardon [208], and Clarens [209]. Production systems were compared in terms of the net energy ratio (NER) of biomass production. NER is defined here as the sum of the energy used for cultivation, harvesting and drying, divided by the energy content of the dry biomass. According to the results of this comparison he found NER less than 1 for six raceway ponds out of eight. Hence a positive energy balance may be achievable for these systems, although this benefit is marginal in the normalized case. The NER of the PBR systems are all greater than 1. The best performing PBR is the flat-plate system which outperforms the tubular PBRs as it benefits from a large illumination surface area and low oxygen build-up [44]. He also found that for raceway ponds the *base case* biomass production cost is ~1.6–1.8 € $kg^{-1}$ and the *projected case* cost is ~0.3–0.4 € $kg^{-1}$, and for idealized tubular PBR the *base case* cost is ~9–10 € $kg^{-1}$ and the *projected case* cost is ~3.8 € $kg^{-1}$ [44]. Raceway pond systems

**Table A1**
List of startup companies attempting to commercialize algal fuels.

| S.N. | Company/location | Country | Website |
|---|---|---|---|
| **01** | Algenol Biofuels, Bonita Springs, FL, Fort Meyers | USA | www.algenolbiofuels.comhtpp |
| **02** | Aquaflow Bionomics, Nelson | New Zealand | www.aquaflowgroup.com |
| **03** | Aurora Algae Inc., Hayward CA | USA | www.aurorainc.com |
| **04** | Algae Link Roosendaal | Netherlands | www.algaelink.com |
| **05** | Aquatic Energy, LLC, Lake Charles Louisiana | USA | www.aquaticenergy.com |
| **06** | ALG Western Oil | South Africa | www.algbf.co.za |
| **07** | Alga Fuel, S.A., Sines | Portugal | www.a4f.pt |
| **09** | A2BE Carbon Capture, Boulder Colorado | USA | www.algaeatwork.com |
| **10** | Bioalgene, Seatle, WA | USA | www.bioalgene.com |
| **11** | BFS Biopetróleo, San Vicente del Raaspeig | Spain | www.biopetroleo.com |
| **12** | Blue Marble Energy, Seattle, Washington | USA | www.bluemarblebio.com |
| **13** | Bionavitas, Inc, Redmond, WA. | USA | www.bionavitas.com |
| **14** | Bodega Algae, LLC, Boston, MA | USA | www.bodegaalgae.com |
| **15** | Cellana, Hawaii | USA | www.cellana.com |
| **16** | Circle Biodiesel and Ethanol Corp., San Marcos, CA | USA | www.circlebio.com |
| **17** | Community Fuels, Encinitas, CA | USA | www.communityfuels.com |
| **18** | Diversified Energy, Gilbert, Arizona | USA | www.diversified-energy.com |
| **19** | Eni | Italy | www.eni.com |
| **20** | Galp Energia, Lisbon | Portugal | www.galpenergia.com |
| **21** | Global Energy Solutions, Vancouver | Canada | www.globalgreensolutionsinc.com |
| **22** | Green Fuel Technologies, Cambridge, Massachusetts | USA | ⟨www.greenfuelonline.com⟩ |
| **23** | Green Shift Corp, New York | USA | www.greenshift.com |
| **24** | HR Biopetroleum, Hawaii | USA | www.hrbiopetroleum.com |
| **25** | Ingrepo B.V, Zutphen | Netherlands | www.ingrepro.nl |
| **26** | International Energy, Vancouver | Canada | www.internationalenergyinc.com |
| **27** | Inventure Chemical, Seattle | USA | www.inventurechem.com |
| **28** | LiveFuels, Inc., San Carlos, CA | USA | www.livefuels.com |
| **29** | Mighty Algae Biofuels, CA | USA | |
| **30** | Neste Oil, Helsinki | Finland | www.nesteoil.com |
| **31** | Origin Oil Inc., Los-Angeles, California | USA | www.originoil.com |
| **32** | OilFox S.A | Argentina | www.oilfox.com.ar |
| **33** | Parabel Inc. formerly PetroAlgae Inc., Melbourne, FL | USA | www.parabel.com |
| **34** | PhycoBiosciences, Chandler, AZ | USA | www.phyco.net |
| **35** | PetroSun, Scottsdale, Arizona | USA | |
| **36** | Sapphire Energy, San Diego | USA | www.sapphireenergy.com |
| **37** | SolenaFuels, WA | USA | www.solenafuels.com |
| **38** | Solix Biofuels, Fort Collins, Colorado | USA | www.solixbiofuels.com |
| **39** | Solazyme, Inc., San Francisco | USA | www.solazyme.com |
| **40** | Seambiotic, Ashkelon | Israel | www.seambiotic.com |
| **41** | Sartec Anoka, Minnesota | USA | www.xlrenewables.com/ |
| **42** | Solarvest BioEnergy | Canada | www.xlrenewables.com |
| **43** | XL Renewables, Phoenix, Arizona | USA | www.xlrenewables.com |

demonstrate a lower cost of algal biomass production than photobioreactor systems. Most of the production costs in raceway system are associated with operation (labor, utilities and raw materials). The cost of production in PBRs, in contrast, is dominated by the capital cost of the PBRs [44].

Another recent study done by Jonker [210], regarding the energy consumption ratio and overall bio-energy production costs of micro-algae cultivation, harvesting and its conversion to energy products, gives an insight for future perspectives of micro-algae production for energy purposes in three different climate profiles of Spain. Jonker [210] covered three phases of total chain (cultivation, harvesting and conversion) in the economic evaluation which resulted in total production costs of heat, fuels and electricity derived from micro-algae. He found that the lower end of fuel production cost calculated for raceway ponds is 136 € 2010/GJ and 153 €2010/GJ for horizontal tubular systems which is very much greater than gasoline–diesel 5–20€ 2010/GJ [210]. He found low end of cultivation costs that is 31€ 2010/GJ and 59€ 2010/GJ for raceway and horizontal tubular PBR respectively. And the overall production costs with cost reduction measures are 65, 105, 111 € /GJ for heat, fuel and electricity respectively with raceway ponds, and for horizontal tubular they are 72, 116 and 122 €/GJ [210]. Almost all the LCA studies recommend more technology advancements to make algae fuel commercially viable.

## 7. Conclusion

In this work we have reviewed and presented the progresses in the production technologies for making microalgae based liquid fuels. Outcomes of different technologies used for cultivation, extraction and biofuel production via diverse routes from promising microalgae strains were investigated to see the evolution in this field for future perspective.

The immense potential of microalgae for producing environmentally sustainable transport fuels is the main motivation behind their development and is receiving support from R&D and investors around the world. This is due to their high biomass productivity, readiness for harvest and high lipid contents (20–75 wt%) than other terrestrial crops. In this review we have covered the best suitable biofuel microalgae strains; among those few are *B. braunii*, *Nannochloropis sp*, *C. vulgaris*, *C.a minutissima*, *C. protothecoides, C. emersonii*, *S. platensis*, *S. maxima*, *D. tertiolecta*, *P. tricornutum, S. obliquus*, *Chlorococcum sp*, *Crypthecodinium cohnii*, *C. reinhardtii*, *Schizochytrium sp*, *D. salina*, and *Microcystis aeruginosa.* These strains are investigated heavily in the literature for fuel purpose due to their higher lipid contents and ability to yield biodiesel, biohydrogen, biohydrocarbons and biomethane. However, these strains still need more ideal culture and growth conditions to obtain algal biomass cheaply. This review also reveals the associated cultivation systems for these strains. Closed photobioreactor configurations seem more promising than open ponds or raceways to meet the need of biofuel industries. Different types of PBRs have been developed over the past decades and T-PBR, FP-PBR and bubble column have shown optimum biomass concentrations. Photobioreactor designs are evolving rapidly and efforts are being made to improve light distribution, mass transfer, shear stress and other PBR operations to make them highly efficient for commercial use.

This study also underlines the various methods to extract the algal oil. The solvent based route for different microalgae species has been used extensively and is believed to be most suitable on a large scale. An environmentally friendly technique like SC-$CO_2$ and non-solvent based technologies like microwaves, pulse electric filed and ultrasonic are still in the developmental stage and require detailed R&D for industrial scale processes.

Microalgae are an important source of biomass. It has been reviewed that algae biomass can be used to produce biodiesel, biohydrogen, bio-oil, bio-ethanol and biomethanol using thermochemical/hydrothermal liquefaction and gasification processes. Most of the literature reports the advantage of the use of catalysts in the bio-crude production from microalgae. Both homogeneous and heterogeneous catalysts systems have been used in hydrothermal liquefaction of microalgae. Current developments in biofuel catalyst systems for pyrolysis and liquefaction are promising; new catalytic processes may allow the production of gasoline, diesel, and jet fuel that is more economical and competitive than existing fuels. However, many issues are still unclear and require further research to be used on industrial scale. Biohydrogen from microalgae as a clean future energy carrier has also been reported in this study and this also needs more innovative research to improve photo-conversion efficiencies by improving culture conditions and effectiveness of photobioreactors.

## Appendix A

See Table A1.

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